Filamentous Moulds Associated with Some Traditionally Prepared Starter Cultures of North East India

A Thesis Submitted

To

Sikkim University

For the Degree of Doctor of Philosophy

By

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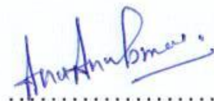
JUNE 2020

Dedicated to
my parents

Smt. Sangita Sinha
&
Sri Devendra Kumar Sinha

DECLARATION

I declare that the present Ph.D thesis entitled **"Filamentous Moulds Associated with Some Traditionally Prepared Starter Cultures of North East India"** submitted by me for the award of the degree of **Doctor of Philosophy in Microbiology** of Sikkim University under the supervision of **Professor Dr. Jyoti Prakash Tamang,** Professor, Department of Microbiology, School of Life Sciences, Tadong, Sikkim University, is my original research work solely carried out by me in the Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok. No part thereof has been submitted for any degree or diploma in any University/Institution.



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This is to certify that the PhD thesis entitled **"Filamentous Moulds Associated with Some Traditionally Prepared Starter Cultures of North East India"** submitted to **SIKKIM UNIVERSITY** in partial fulfilment for the requirement of the Doctor of Philosophy in Microbiology, embodies the work carried out by **Ms. Anu Anupma** for the award of PhD Degree in Microbiology, Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, Sikkim. It is a record of bonafide investigation carried out and completed by her under our supervisions and guidances. She has followed the rules and regulations laid down the University. The results are original and have not been submitted anywhere else for any other degree or diploma.

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"Filamentous Moulds Associated with Some Traditionally Prepared Starter Cultures of North East India"

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ACKNOWLEDGEMENTS

The long journey of my doctoral research is totally incomplete without help and support of several people who came through this life-changing experience and made my journey comfortable and smooth. I humbly take this opportunity to acknowledge them whose eternal support enable me to complete my research work into final doctoral thesis.

First and foremost, I would like to say a very big thank you to my supervisor, Professor Dr. Jyoti Prakash Tamang, Department of Microbiology, Sikkim University to whom I owe my entire efforts as without his keen supervision, able guidance, academic co-operation and invaluable suggestions, this work would not have been possible. I am deeply indebted to him for his kind gesture. I want to thank him for all his support and true mentorship which was never restricted to my thesis only but also to my development.

Besides my supervisor, I would also like to extend my sincere gratitude to all the faculty members of Department of Microbiology, Dr. Hare Krishna Tiwari, Associate Professor and Head of Department, Dr. Buddhiman Tamang, Dr. Bimala Singh, Dr. Nagendra Thakur and Dr. Anil Kumar Verma, Assistant Professors of Department of Microbiology, Sikkim University for their continuous support and guidance.

I take this opportunity to acknowledge my sincere gratitude to Prof. Avinash Khare Vice-Chancellor, Sikkim University for his encouragement and support and establishing a central university in Sikkim which is providing an ideal atmosphere for many students to pursue their career. I am also very much thankful to Prof. M.P. Lama as well as Prof. T.B. Subba former Vice-Chancellors of Sikkim University for their suggestions and blessings.

I would like to thank Mrs. Radha Basneet (Laboratory in charge, Department of Microbiology), Shri Pukar Bishwakarma and Shri Gagan Sen Chettri for their help and support in technical assistance.

I would like to thank Department of Microbiology, School of Life Sciences, Sikkim University, for providing excellent working environment, lab spaces and equipment for my research work.

I would also like to thank **Bioinformatics Centre of Sikkim University DAICENTER**, for providing excellent working environment, lab spaces and equipments for my research work.

I wish to express my warm and sincere thanks to Dr. Namrata Thapa Tamang, Head, Department of Zoology, Sikkim Government College, Tadong, Gangtok, for her constant support and blessing during my Ph.D. work.

I would like to thank UGC, Govt. of India, for providing me with the most essential funding (UGC Non-NET fellowship) for my research work. I also take this opportunity sincerely to acknowledge the assistance of the **Bioinformatics Centre of Sikkim University DAICENTER for my student fellowship.**

I am thankful to Botanical Survey of India, Gangtok, Sikkim branch for identifying our plant samples.

I would like to thank Professor Karuna Shrivastava, Department of Biotechnology, North Eastern Regional Institute of Science and Technology, Nirjuli. Arunachal Pradesh for her help during samples collection.

I would like to express my sincere gratitude to Dr. Rajesh Kumar, Scientist and Dr. Sanjay Singh NFCCI, Pune, for their technical help and support during my Ph.D. research work.

Sincere thanks are due to Rakesh Kumar Ranjan, Assistant Professor, Department of Earth Sciences, Sikkim University for his guidance and support.

I would like to thank Prof. A.S. Chandel, Librarain and Shri Pranab Kumar Sarkarl Controller of Examinations, Sikkim University for their help and support.

I am thankful to all ethnic people of our North East India, who have vast native skills and wisdom, for sharing their indigenous knowledge on food fermentation to us.

I am thankful to my Senior Mrs. Pramila Koirala, Mrs. Kriti Ghatani, Mrs. Varsha R Gajamer, Mrs. Bhumika Poudyali, Mr. Ranjan Kausal Triwa, for their help and support during my Ph.D. work.

Words cannot convey what I owe to my batchmates, Sayak Das, Mingma T Sherpa, Lalit K Chaurasia, Nilu Pradhan and Ashish Kumar Singh for their untiring help whenever need arises and without whom this would have been an uphill task.

Also, my DAICENTER team was always with me during my best and worst of times. I would like to express my heartfelt acknowledgement to Ms. Ranjita Rai, Ms. Meera Bhutia,

Mrs. Pooja Pradhan, Mr. Nakibapher Jones Shangpliang, Mr. Pynhunlang Kharnaior, Ms. Priyambada Pariyar, Mr. Souvik Das and Dr. Shankar Prasad Sha.

I would like to express my sincere gratitude to Dr. Mangesh V. Suryavanshi, for their technical help and support during my PhD research work.

My heartiest thanks go to my dearest friends Amrita Singh, Beej Gandhi, Sapna Kumari, Pallavi Kumari, Megha Paul, Archana Kumari and Manjeet Kumar for their care and support during my whole Ph.D. journey.

I am indebted to my parents, Smt. Sangita Sinha and Sri Devendra Kumar Sinha for not imposing their wishes on me; rather they stood behind me in favour of my decisions, without thinking twice of the repercussions. They have always been continuous source of encouragement and motivation for me at every stage of my life. I sincerely would like to express my deepest love to my brother Anurag Raj and sister Anjali Rani whose keen help and encouragement supported me at every stage.

I am extremely pleased and grateful to my husband Mr. Sanjeev Kumar for his unconditional love, care and support and who has been by my side, living every single minute of it, and without whom, I would not have had the courage to embark on this journey in the first place.

I owe a deep debt of gratitude and respect to my father-in-law (Late Hareram Singh) and mother-in-law (Mrs. Malti Devi) and all the family members for extending their affection and support.

Last but not least I would like to acknowledge the assistance of Sikkim University for providing me UGC Non-NET fellowship for my Ph.D. work and also to Bioinformatics Centre of Sikkim University DAICENTER for student fellowship.

I will always remain thankful to all those who directly or indirectly connected with my research work. It would not have been possible for me to present my research work as the full scientific doctoral thesis without their active help. This research work of mine is also the product of the god and his blessing which kept me able to work hard physically and mentally and overcome many hurdles in this doctoral thesis journey. I owe the god this day for making my dream come true which I have been living since my childhood.

Date: 22nd June 2020 **Anu Anupma**

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INTRODUCTION

Fermented beverages and alcoholic drinks represent a vast diversity of alcoholic products ranging from ethnic fermented beverages, distillate products to wine and beer (Marsh et al. 2014; Tamang et al. 2020). Making of alcoholic beverages are widespread interests to enhance the pleasure of drinking and entertainment (Savic et al. 2016) and have nutritional values and health benefits (Dunbar et al. 2017). Ethnic alcoholic beverages have strong ritualistic importance among the ethnic people in Asia (Tamang 2016), Africa (Ezekiel et al. 2018), Latin America (Pierce and Toxqui 2014) and Europe and North America (Peele 1997) where social activities require provision and drinking of appreciable quantities of alcohol (Tamang et al. 2015). Alcoholic beverages are among the most ethnically and culturally important fermented food products in the world which are associated with many socio-culturally-accepted practices, including rituals, customs, religions including worship and entertainment (Tamang et al. 2020). Wine, an alcoholic drink made from fermenting grapes (Belda et al. 2017), has a long historical and socio-cultural significance in food habits of European and Mediterranean populations (Tamang and Samuel 2010). In contrast, wine is a not traditional alcoholic drink in Asia, where fruits including grapes are generally consumed directly without fermentation (Tamang et al. 2016a). Instead, alcoholic beverages/drinks in Asia are usually made from cereals and potatoes/cassava which contain few fermentable carbohydrates (Blandino et al. 2003) by traditionally prepared dry starters which are used to convert starches to sugars (Hesseltine et al. 1988; Tamang et al. 2016a). Thus, an enzyme-mediated saccharification step is necessary, commonly by filamentous moulds and amylase-producing yeasts (Tamang and Fleet 2009; Aidoo and Nout 2010), and then glucose to alcohol production mostly by alcohol-producing yeasts (Walker and Stewart 2016) and filamentous fungi (Skory et al. 1997). Traditional beer-making by malting process for alcohol production is rare or known in Asia (Tamang 2016).

Even, historically and culturally, wine, whisky, rum, gin and brandy were not traditional alcoholic beverages of Asia until colonial rule influences the drinking habit in Asia (Tamang et al. 2020).

Historically preparation and drinking of alcoholic drinks in India have originated since Indus Valley civilization traced back to 8000 years (Sarkar et al. 2016) mostly through fermentation (Singh et al. 2010) and distillation (Achaya 1991), based on evidences of clay pots items from excavations sites (Mahdihassan 1979). During *Vedic* (1500 BCE) in India, distilled liquor prepared by fermenting millets known as *sura* and also another alcoholic product prepared from fermented flowers called *parisrut* were considered as the ancient Indian alcoholic beverages (Mahdihassan 1981). No historical records on vinification (fermentation of grapes into wine), malting and brewing (such as beer) processes have been recorded in Indian dietary culture (Tamang 2020). Traditional beverages and alcoholic drinks are prepared either by spontaneous fermentation of plant or cereals, or by using traditionally prepared dry starters in India (Tamang 2010b). Wine, beer, whisky is not traditional drink of India though these alcoholic drinks are becoming popular in modern Indian food culture (Tamang 2020).

The geographical locations of the Indian Himalayas are categorized into Western, Central, Eastern and Purvanchal Himalayas (Nandy et al. 2006). The Eastern Himalayan region of India lies between the latitudes 26° 40'-29° 30' North and longitudes 88° 5'-97° 5' East and covers a total area of 93,988 km^2 comprising Darjeeling hills, Sikkim, and Arunachal Pradesh. The Purvanchal Himalayas lie between the latitudes 21° 5' - 28° 23' North and longitudes 91° 13' - 97° 25' East, covering a total area of 108, 229 km^2 comprising hills of Assam (15, 322 km^2), Manipur (22,327 km^2), Meghalaya (22,429 km^2), Mizoram (21,081 km^2), Nagaland (16,579 km^2), and Tripura (10,491 km^2). North East states of India consist of eight-state viz.

Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. The regions of North East India have international boundary in the east with Myanmar, south and west with Bangladesh, north-west with Bhutan and north with Tibet Autonomous Region of China. Multi-ethnic diversity is prevalent among the populations of North East India where about 225 of India's 470 ethnical communities live-in North-East India (Das and Deka 2012) representing 75% of the population of North East India (Agrahar-Murugkar and Subbulakshmi 2006). The major ethnic groups living in North East India are summarized as follows (Tamang 2010b; Gupta 2018): **Arunachal Pradesh** (Monpa, Sherdukpen, Memba, Khamba, Khampti, Singpho, Adi, Aka, Apatani, Bangni, Nishing, Mishmi, Miji, Tangsa, Nocte, Wancho); **Assam** (Ahom, Bodo, Karbi, ethnic Nepali, Miri, Rabha, Bengali); **Manipur** (Meiti, Kuki, ethnic Gorkha); **Meghalaya** (Khasi, Garo, Jaintia, ethnic Nepali/Gorkha); **Mizoram** (Mizo, Hmar, ethnic Gorkha, Lakher, Pawi); **Nagaland** (Angami, Chakhesang, Ao, Sema, Rengma, Lotha, Chang, Konyak, Sangtam, Phom, Zeliang, Mao, Maram, Tangkhul, Maring, Anal, Mayao-Monsang, Lamkang, Nockte, Haimi, Htangun, Ranpan, Kolyo, Kenyu, Kacha, Yachimi, Kabui, Uchongpok, Makaoro, Jeru, Somra, ethnic Gorkha); **Sikkim** {Ethic Gorkha/Nepali (Rai, Limboo, Tamang, Bahun, Chettri, Magar, Pradhan/Newar, Gurung, Bhujel, Dewan, Sansyasi/Giri, Sunwar, Khagatey, Sherpa, Kami, Damai, Sarki, Maji), Lepcha, Bhutia} and **Tripura** (Chakmas, Bengali).

Due to their livelihood in hilly terrains, the people of North East have rich traditional knowledge of food production from locally available raw materials (Tamang et al. 2012). Ethnic people of North East India have deep-rooted association with nature for livelihood to whom they also worship and preserve nature by birth (Singh and Singh 2007). Some ethnic people of India traditionally prepare amylase and alcohol-

producing starters to ferment alcoholic beverages for home consumption, which are known by different vernacular languages spoken locally in the regions such as *marcha* in Sikkim and Darjeeling hills, *thiat* in Meghalaya*, humao* in Assam, *hamei* in Manipur*, chowan* in Tripura*, phut* in Arunachal Pradesh, *dawdim* in Mizoram and *khekhrii* in Nagaland (Anupma et al. 2018). Traditional methods of preparation of dry starters are almost same in North East India with some differences in use of starch-rich substrates such as rice or wheat or barley and wrapping materials either fern fronds or dry paddy-straws or fresh leaves of locally available wild plants (Shrivastava et al. 2012). Soaked, dewatered and ground cereal (rice/wheat/barley) flours are mixed with some wild plants, few spices such as sun-dried chilies, garlics and supplemented with 1-2% of previously prepared dry starters in powder forms ("back-slopping method" for sub-culturing the microbiota) to make thick doughs with addition of water. Dough mixtures are shaped accordingly into flattened to ball-types of variable sizes, then placed on fresh ferns or other plant leaves/dry paddy straws and allowed to ferment under semi-anaerobic conditions for 2-3 days at room temperature inside the room. After desirable fermentation, fermented doughs are then sun dried for 2-3 days to obtain dry starters which are exclusively used to ferment cereals into mild/strong alcoholic beverages (Tamang 2010a). However, *khekhrii,* a dry starter of Nagaland in India is prepared by naturally fermenting sprouted-rice grains which are then dried in the sun to obtain dry granules of starter to prepare alcoholic beverage locally called *zutho* (Anupma et al. 2018). Dry starters of North East India are similar amylase and alcohol-producing starters of other states of India such as *dhehli, balam, maler, treh* and *bakhar* of Himachal Pradesh and Uttarakhand (Thakur et al. 2015), *ranu dabai/goti* of West Bengal, Odisha and Jharkhand (Ghosh et al. 2015), *apop pitha or modor pitha* of Assam (Barooah et al. 2020), *keem, malera/treh* of Himachal Pradesh (Kanwar and Bhushan

2020) and other Asian countries such as *nuruk* of Korea (Jung et al. 2012), *daqu* of China (Zheng et al. 2012), *benh men* of Vietnam (Dung et al. 2007), *ragi* of Indonesia (Roslan et al. 2018), *bubod* of the Philippines (Fronteras and Bullo 2017), *loogpang* of Thailand (Daroonpuntet et al. 2016) and *dombea* or *medombae* of Cambodia (Ly et al. 2018).

Microbiology of some Asian cereal-based dry starters have been studied earlier and reported species of filamentous moulds (Hesseltine et al. 1988; Hesseltine 1991; Yang et al. 2011; Lv et al. 2012a; Chen et al. 2014; Das et al. 2017; Tang et al. 2019; Liu et al. 2020), yeasts (Hesseltine and Kurtzman 1990; Tamang and Sarkar 1995; Tsuyoshi et al. 2005; Thanh et al. 2008; Jeyaram et al. 2008, 2011; Fronteras and Bullo 2017; Sha et al. 2016, 2017, 2018, 2019) and bacteria (Hesseltine and Ray 1988; Tamang et al. 2007; Sha et al. 2017; Roslan et al. 2018; Pradhan and Tamang 2019) are found to be coexisted in traditionally prepared dry starters for alcohol production which have been "sub-cultured" in crude manner, probably to preserve essential microbiota and mycobiota by ethnic people of Asia for hundred years. Filamentous moulds are also responsible for the qualities of alcoholic beverages including nutritional values and sensory properties such as flavor, taste and color (Zhang et al. 2015; Tamang et al. 2016b). Though studies on some dry starters of India were conducted earlier and even information on composition of yeasts and bacteria have been reported (Tamang et al. 2016c) but very limited information on taxonomy of filamentous moulds are available, except in *marcha* (Hesseltine 1991; Tamang et al. 1988; Tamang and Sarkar 1995; Sha et al. 2017; 2019), *thiat* (Sha et al. 2017; 2019), *amou* and *perok-kushi* (Das et al. 2017). *Mucor circinelloides*, *Rhizopus chinensis* and *Rhizopus stolonifer* were reported earlier from *marcha* samples collected from Nepal, Darjeeling and Sikkim (Tamang et al. 1988; Tamang and Sarkar 1995; Tamang and Thapa 2006; Sha et al. 2017, 2018),

Amylomyces rouxii and *Rhizopus oryzae* from samples of *amou* and *perok-kushi*, traditional starters of Assam (Das et al. 2017).

Fungal species in this earth are estimated to be 1.2 million (Wu et al. 2019) out of which only 7% were identified with an extinction rates of 5% per decade (Costello et al. 2013). Hence, isolation and identification of fungi by molecular technique is essential to profile and preserve the unexplored fungal diversity in different eco-systems including foods (Hyde et al. 2019). Accurate taxonomical identification to species level is a reliable key for fungal diversity analysis (Tedersoo et al. 2018). Filamentous moulds have generally been identified and classified on the basis of morphology of spores and spore producing structures (Samson et al. 2004; Hibbett et al. 2007; Kirk et al. 2013). In addition, sequence-based taxonomy supported by machine learning tool (Krachunov et al. 2017) has revolutionized our understanding of phylogenetic relationships among the fungi (Hibbett et al. 2016; Tedersoo et al. 2018).

Filamentous moulds belong to Ascomycetes have gained great importance especially as producers of antibiotics and in food production such as baking, brewing and fermentation (Dupont et al. 2017; Drozłowska 2019). Ascomycota and Basidiomycota are known as efficient producers of extracellular enzymes (Sajith et al. 2016). Species of *Aspergillus, Penicillium, Mucor, Cladosporium* and *Rhizopus* play an important role in the production of various food and beverages (McKelvey and Murphy 2011; Lv et al. 2015). *Aspergillus* species are commonly used in large-scale fermentation in the production of alcoholic beverages, enzymes (a-amylase, xylanase, and cellulose), organic acids, and bioactive compounds (Cairns et al. 2018). *Penicillium* species are well known for secretion of lipase, glucose oxidase, xylanase which is used in production of antibiotic and cheese production (Dupont et al. 2017; Raveendran et al. 2018). Filamentous moulds belong to Basidiomycota have biotechnological and

environmental application mostly for extracellular enzymes (cellulase, hemicellulose, laccase and xylanase), synthesis of flavour and dye (Songulashvili et al. 2007; Schmidt-Dannert 2016). Zygomycetes fungi also produce amylase and alcohol during the fermentation process which include *Amylomyces, Actinomucor, Mucor* and *Rhizopus* (Karimi and Zamani 2013). Some filamentous moulds are involved in extracellular enzymatic activities (Huang et al. 2017) and also have antinutritive degrading abilities in the fermentation (Jatuwong et al. 2020). Filamentous moulds in dry starters have been known to produce amylolytic and proteolytic enzymes that degrade starch and proteins of raw materials during the fermentation (Cho et al. 2012; Carroll et al. 2017).

Since traditionally prepared dry starters of Asia are consortia of all major groups of organisms consisting of mycelial moulds, yeasts and bacteria (Jung et al. 2012; Huang et al. 2017; Ly et al. 2018; Gan et al. 2019; Liu et al. 2020). Each major group has specific roles in alcoholic fermentation (Thapa and Tamang 2004; Tamang and Thapa 2006; Nile 2015; Kiefer et al. 2018; Zou et al. 2018; Wang et al. 2019). Moreover, some reports on yeasts composition in dry starters of North East India (Tamang and Sarkar 1995; Tsuyoshi et al. 2005; Jeyaram et al. 2008, 2011; Chakrabarty et al. 2014; Sha et al. 2018, 2019) and also on bacterial composition (Tamang et al. 2007; Pradhan and Tamang 2019) are available, but limited information on composition of filamentous moulds in starters of North East India are available (Tamang et al. 1988; Tamang and Sarkar 1995) purely based on morphological characters. Morphological characterizations of fungi are useful for identification up to the family or genus level (Alsohaili and Bani-Hasan 2018), however, this method is not adequate to identify the fungi up to species level (Lutzoni et al. 2004). Nowadays, sequence-based taxonomy tool is widely applied to confirm the exact identification of the fungal species

(Romanelli et al. 2010; Xu 2016). Hence, the present Thesis is aimed to identify the filamentous moulds isolated from eight different types of traditionally prepared starters of North East India viz. *marcha* of Sikkim, *thiat* of Meghalaya, *humao* of Assam, *hamei* of Manipur, *chowan* of Tripura, *phut* of Arunachal Pradesh, *dawdim* of Mizoram and *khekhrii* of Nagaland to species level by morphological characters (macroscopic and microscopic) and molecular identification using internal transcribed spacer (ITS) gene sequencing method. The Thesis is also aimed to illustrate the Taxonomical Keys to fungal species which may help the researchers to refer the Taxonomical Keys to study fungal species from other eco-systems elsewhere. We believe this is the first report on illustration of Taxonomical Keys of fungal species from starters of North East India. Lastly, the Thesis is aimed to analyse some enzymatic activities and screening of antinutritive degrading enzymes.

Objectives:

To understand the filamentous moulds diversity and their enzymatic activities in some traditionally prepared dry starters of North East India, the present Thesis focused on the following Objectives:

1) To collect samples of traditionally prepared starter cultures of North East India for isolation of filamentous moulds.
2) To study the phenotypic characterization and identification of filamentous moulds.
3) To study the genotypic characterizations of moulds isolates.
4) To determine some enzymatic activities and anti-nutritive degrading factors producing abilities of filamentous moulds.

REVIEW OF LITERATURE

Alcoholic beverages

Indigenous fermented foods and alcoholic beverages have been a part of human diet since the Indus valley civilization dated back to 8000 years ago (Anal 2019; Tamang 2020). Fermented food is generally produced by using plants or animal ingredients in combination with yeast, filamentous moulds or bacteria which are either sourced from the environment or carefully kept in cultures maintained by humans (Scott and Sullivan 2008; Slivinski et al. 2011). Fermentation is one of the most primordial and economical techniques of food preparation and preservation in the world (Caplice and Fitzgerald 1999; Nuraida 2015). Basically, fermented foods and alcoholic beverages are defined as products made by aerobic and anaerobic microorganisms which include yeast, moulds, bacteria or a combination of all of them (Campbell-Platt 1987; Ansorena and Astiasaran 2016; Sanlier et al. 2019). These microorganisms have an important role in the enzymatic conversion of major and minor food constituents (Gille et al. 2018; Sanlier et al. 2019), which help to change the organoleptic characteristics of foods through developing a wide diversity of the flavours, aromas and textures (Sanlier et al. 2019; Walsh et al. 2016). Besides organoleptic properties, fermentation also improves digestibility, nutritional quality and exert health promoting benefits through enrichment of food substrates with vitamins, proteins, essential amino acids and essential fatty acids (Steinkraus 1994, 1997, 2002; Nout and Motarjemi 1997; Giraffa 2004). Moreover, fermentation may provide assistance in the destruction or detoxification of certain undesirable compounds which may be present in raw foods (Mota de Carvalho et al. 2018). Fermented foods are encountered worldwide and their origin are due to their prolonged shelf life, reduced volume, shorter cooking times and superior nutritive value as compared to the non-fermented food ingredients (Rolle and Satin 2002; Joshi 2016). The traditional way of carrying out fermentation at the household-scale is still followed

using relatively simple processing facilities (Holzapfel 1997; Joshi 2016). These products often contain mixed microbial populations because of the lack of sterility and the use of natural fermentation (Nout and Sarkar 1999; Das and Deka 2012). The indigenous fermented foods are food substrates that are overgrown by edible microorganisms as starter (Mani 2018). These mixed starter cultures are used to convert starchy materials to sugar and subsequently to alcohol and organic acids (Hesseltine et al. 1988; Nehal 2013).

Fermented beverage is one of the ancient fermented food first reported in 5000 BCE in Babylon, 3150 BCE in Ancient Egypt, 2000 BCE in Mexico and 1500 BCE in Sudan (Tamang and Samuel 2010). Most ancient alcoholic beverage is *pulque* (*Agave* juice) from South America. Fermented alcoholic beverages have been widely consumed since pre-Vedic times in Indian history (2500-200 BCE) (Tamang and Samuel 2010; Tamang 2020), which have been mentioned in Holy book such as *Ramayana* (300-75 BCE) (Prakash 1961) and *Bhagavad Gita* (Tamang et al. 2015; Tamang et al. 2016c). Based originally during the period of the Indus river system, wine was worshiped as the liquid God named Soma, because of its nutritional attributes (Tamang et al. 2016b). According to Vedas, Soma is awarded with great medicinal power (Tamang et al. 2015). Initial perception which is made about Soma that he used to have the fermented juice of an East Indian leaf-less wine (*Sarcostemma acidum*) and supplementary wild indigenous grape wines (Hui et al. 2012). The malting process as well as the process of wine fermentation is unknown in traditional fermentation processes in Asia resulting amylolytic mixed starters prepared from the growth of moulds and yeasts on raw or cooked cereals are more commonly used (Tamang 2020). The use of traditionally prepared amylolytic mixed starters is common to the Himalayas and the South East Asia (Tamang et al. 2015). In Asian country, traditional ways of sub-culturing the

essential microorganisms (consortia of filamentous moulds, yeasts and bacteria) with rice or wheat as the carbon-source based, in the form of dry, flattened or round balls, for making of alcoholic beverages are innovative indigenous technology (Haard et al. 1999; Tamang 2016b). A starter culture is basically defined as a preparation used in the production of a fermented food that contains a large number of variable microbial cells or at least one microorganism that can be added to bring about desirable changes in a food substrate (Vogel et al. 2011; Caballero et al. 2003) and to accelerate a fermentation process (Holzapfel 2002; Leroy and Vuyst 2004). It allows for more strict control of a fermentation process, the outcome of which may therefore be predictable (Hesseltine et al. 1988; Smid et al. 2014; Bachmann et al. 2015). Hence, information of microbial diversity in a starter culture is essential to improve its quality and safety, for culture selection and enhancement of nutritional value of fermented food and alcoholic beverages (Roslan et al. 2018).

Table A: Ethnic fermented beverages and alcoholic drinks with amylolytic starters of the world

Sl. No.	Amylolytic starters	Examples
1.	Non-distilled and unfiltered alcoholic beverages	*Lao-chao* of China, *tapé* of Indonesia, *makgeolli* of Korea (fermented rice), *bhaati-jaanr* (fermented rice) and *kodo ko jaanr* (fermented finger millets) of India and Nepal (Tamang 2006), *kanji* of India (Tamang 2012a) and makgeolli (fermented rice) of Korea (Jung et al. 2012).
2.	Non-distilled and filtered alcoholic beverages	*Saké* of Japan (Kotaka et al. 2008)
3.	Distilled alcoholic beverages	*Shochu* of Japan, and *soju* of Korea (Steinkraus 1996).
4.	Alcoholic beverages produced by involvement of amylase in human saliva	*Chicha* of Peru (Calle-Vallejo and Koper 2013).
5.	Alcoholic beverages produced by mono- (single-strain) fermentation	Beer (Alcohol content 2-8%) (Kurtzman and Robnett 2003).
6.	Alcoholic beverages produced from honey	*Tej* of Ethiopia (Bahiru et al. 2006).
7.	Alcoholic beverages produced from plant parts	*Pulque* of Mexico (Lappe-Oliveras et al. 2008), *toddy* of India (Singaravadivel et al. 2012) and *kanji* of India (Hui et al. 2012).

8.	Alcoholic beverages produced by malting (germination)	*Sorghum* (“*Bantu”)* beer of South Africa (Kutyauripo et al. 2009), *pito* of Nigeria and Ghana (Kolawole et al. 2013), and *tchoukoutou* of Benin (Greppi et al. 2013).
9.	Alcoholic beverages prepared from fruits without distillation	Wine, cider
10.	Distilled alcoholic beverages prepared from fruits and cereals	Whisky and brandy (Spaho 2017)

The amylolytic starter culture originated during the time of Euchok, who was the daughter of the legendary king of Woo of China and also known as the Goddess of rice-wine in Chinese culture in 4000 BC (Lee 1984; Lee and Kim 2016). Chinese amylolytic starter named as *chu* was first reported in Shu-Ching document written during Chou dynasty (1121-256 BCC) in which it is reported that *chu* is essential for making alcoholic beverages (Haard et al. 1999).The *chu* is very similar to *marcha* of the Eastern Himalayan region which is used in preparation of alcoholic beverages named *bhaati jaanr* (Tamang and Thapa 2006).

The drinking of alcoholic beverage prepared from starter culture is a common exercise between many ethnic communities residing in the North-East India (Tamang et al. 2020). It plays an important role in the sociocultural life of the ethnic people as it is found to be associated with many occasions like merry-making, ritual ceremonies, festivals, marriages and even death ceremonies (Das et al. 2012a). The preparation and consumption of this type of beverages emerged mainly due to the climatic conditions and discovering the use of surroundings of natural resources (Singh and Singh 2006). The preparation process of ethnic starter cultures which is used to prepare alcoholic beverages in the North East India is usually kept as a secret and the indigenous knowledge of processing is not easily passed on (Tamang 2016). *Marcha* is prepared

by Limboo and Rai of Nepali/Gorkha community, *loogpang* by the *Thai, ragi* by the Indonesians, *nuruk* by ethnic Korean, and *bubod* by ethnic Philippines (Tamang 2020). Traditionally, Asian ethnic people prepare three main types of mixed amylolytic starters to convert cereal starch into sugars and then into alcohol and organic acids in Asia (Table: B) (Tamang 2012; Anal 2019; Sha et al. 2019).

Table B: Three major types of amylolytic starters prepared by Asian ethnic people (Steinkraus 1983; Hesseltine et al. 1988; Tamang and Fleet 2009; Tamang 2010a; Tamang 2020).

Type of amylolytic starters	Organisms	Uses
Type I	Consortia of yeasts, moulds and bacteria	*Marcha* in India and Nepal, *ragi* in Indonesia, *bubod* in Philippines, *chiu/chu* in China and Taiwan, *loogpang* in Thailand, *nuruk* in Korea, and *men* in Vietnam (Tamang et al. 1996; Dung et al. 2007)
Type II	*Aspergillus oryzae* and *Aspergillus sojae*	*Koji* in Japan to produce alcoholic beverages including *sake*
Type III	Yeasts and filamentous moulds	Whole-wheat flour with its associated flora is moistened and made into large compact cakes.

Filamentous moulds diversity in amylolytic starter culture

Fungi are the most diverse group of eukaryotic, heterotrophic both single-celled (yeast), multi-cellular, or as a combination of both forms (dimorphic fungi) organisms (McGinnis and Tyring 1996). Fungi are second most species- rich organism, estimated number of fungal species is between 2.2 and 3.8 million in the ecosystem (Hawksworth and Luecking 2017; Wu et al. 2019). But on the basis of high-throughput sequencing data, an approximate of 3.5 to 5.1 million fungal species are existed (Blackwell 2011). Fungi are classified into four groups on the basis of reproductive and molecular

characteristics; Chytridiomycetes, Zygomycetes, Ascomycetes and Basidiomycetes (Whittaker 1969; Hawksworth et al. 1995; Naranjo-Ortiz and Gabaldón 2019). However, the recent phylogenetic classification of fungi into major lineage are Dikarya (Ascomycota, Basidiomycota), Glomeromycota, Chytridiomycota, Neocallimastigomycota, Blastocladiomycota, Kickxellomycota, Microsporidia and Cryptomycota, respectively (Hibbett et al. 2007; Kameshwar and Qin 2019). Fungi have been involved in many purposes such as the production of fermented foods and alcoholic beverages (Dupont et al. 2017) and used in biotechnology industry for production of different compounds like antibiotics, industrial enzymes, organic acids and food industries beverages (Bourdichon et al. 2012; Hyde et al. 2019; Moore and Chiu 2001). The use of fungi in spontaneous fermentation led to increase the shelf-life of the food products (Nout 1995; Nout and Aidoo 2011). Filamentous moulds present in fermented foods and alcoholic beverages are responsible for pleasant flavor, attractive aroma, nutritional values and texture (Laich et al. 2002; Zhang et al. 2015; Tamang et al. 2016b). Filamentous moulds used as a starter culture should fulfil several requirements such as it should not be toxinogenic, an antibiotic and produce any off-flavours (Hui and Sherkat 2005; Leyva et al. 2017). Also, it should be adapted to the food products which produce the appropriate amounts of proteases, lipases and antagonistic against pathogenic or spoilage bacteria characteristics of the fermented product (Geisen 1993; Walker and White 2017). According to Raja et al. (2017) primary taxonomical identification is based solely on morphology i.e.~31%; about 28% of them did not report any method of identification for the fungus from which secondary metabolites were isolated; based on molecular method only 27% fungal species identified (mostly from the internal transcribed spacer (ITS) region) and

combination of both methods (morphology and molecular data) only ∼14% fungal species identified (Samson et al. 2004; Krachunov et al. 2017).

Identification of filamentous moulds based on morphology

For the last 300 years, morphological characters (phenotypic characters), such as spore-producing structures formed as a result of asexual (mitosis) or sexual (meiosis) reproduction have been used to identify, classify and infer fungal phylogenies (Samson et al. 2004; Hibbett et al. 2007; Kirk et al. 2013; Jayasiri et al. 2015; Raja et al. 2017). In fact, morphology-based identification has limitations which lead to incorrect identification, such as, phenotypic relationships as many of them are subject for example to plasticity, parallelism, reversal (homoplasy) (Judd et al. 2002) and needs a high level of identification expertise (Jayasiri et al. 2015).

Molecular identification of filamentous mould

Molecular characters such as DNA sequence-data are primarily useful in providing more distinctive characters that can be statistically analysed to infer phylogenetic relationships (Shenoy et al. 2007). Molecular phylogenetic analysis of fungi has a 15-25-year history (Blackwell 2011; Jayasiri et al. 2015) and is now becoming a vital part of fungal systematics. The internal transcribed spacer (ITS) region and DNA taxonomy using one or multiple sequence alignments is most likely to successfully classify the megadiverse fungi (Hibbett et al. 2011; Hibbett et al. 2016; Tedersoo et al. 2018).

Cereals as a substrate for starters

Cereals are one of the major food crops as well as substrates for fermented food product worldwide (Brandt 2014; Verni et al. 2019). Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, corn or sorghum) are well known in many parts of the World (Blandino et al. 2003; Tamang et al. 2016b; Ogunremi et al. 2017). Cereals are rich in nutrients (amino acids profile of their proteins, enzymes, lipids, minerals, sugars and vitamins) (Das et al. 2012) and used as the substrate for starter culture raw rice (Rai and Subba 2016). Raw rice starch that is uncooked, ungelatinised state is relatively resistance to the activity of most amylolytic enzymes (Xu et al. 2016). It may be hydrolysed by glucosidase from yeast or filamentous moulds (Owens 2014). Non-sticky rice starch is more popular than sticky rice starch (Rai and Subba 2016). Sometimes, millet and maize powders are also used as a substrate (Nuraida and Krusong 2015).

Rationale for beginning of uses of wild plants, herbs and spices in starter cultures

Different herbs and/or spices are usually included in dry starter culture methods, but their roles in starting preparation has not been clearly established (Narzary et al. 2016). Plants used to prepare starter cultures are typically considered to be an inexhaustible source of essential microorganisms (yeasts, moulds and bacteria). Nevertheless, some authors say that leaves of few wild plants serve as an attractive yeast agent– *Saccharomyces cerevisiae* (Nuraida 2015; Nuraida and Krusong 2015). The producers of *marcha* and *ragi* believe that adding wild herbs give the product more flavour and they also believe that adding chilies and ginger will remove devils that may damage the product at some point of preparation (Tamang 2010a; Tamang 2016). This is simply to test the growth of undesirable microorganisms which can inhibit the growth of local

microorganisms of ethnic starters (Dung et al. 2005; Soedarsono 1972) and the addition of sweet herbs is to complement the carbon supply for growing organisms in *marcha* (Tamang 2010a). However, no comprehensive research was conducted on either development of the microorganism populations or the results of spices throughout the preparation of starters (Nuraida and Krusong 2015). It's additionally possible that the little quantity of herbs more gives more some nutrients for the growth of microorganisms (Tamang 2010a) and their presence has been determined to stimulate growth of genus *Aspergillus rouxii* and *Saccharomyces cerevisiae* (Dung et al. 2005).

Traditionally prepared dry starters of Asia and their alcoholic products:

In Asia unique type of dry starters containing consortium of mycelial or filamentous molds, amylase- and alcohol-producing yeasts and lactic acid bacteria (LAB) with rice or wheat as carbon-source base in the form of dry, flattened or round or balls of various sizes and shapes are traditionally prepared to produce various mild to strong alcoholic beverages and distilled liquor (Hesseltine et al. 1988; Tamang et al. 1996, 2016a; Dung et al. 2007; Aidoo and Nout 2010; Tamang 2010a; Jung et al. 2012; Huang et al. 2017; Sha et al. 2018; He et al. 2019; Pradhan and Tamang 2019). Traditional preparation of Asian dry starters is almost same in every region with some differences in use of starch-rich substrates such as rice or wheat or barley and wrapping materials either fern fronds or dry paddy-straw or fresh leaves of locally available wild plants (Shrivastava et al. 2012; Tamang et al. 2016a). Soaked, dewatered and ground cereal (rice/wheat/barley) flours are mixed with some wild plants, few spices such as sun-dried chillies, garlics and supplemented with 1-2% of previously prepared dry starters in powder forms ("back-slopping method" for sub-culturing the microbiota) to make thick doughs with addition of water (Tamang et al. 2020). Thoroughly mixed dough mixtures are made

into round or flat cakes of variable shapes and sizes, placed on fresh ferns or other plant leaves/dry paddy straws and allowed to ferment under semi-anaerobic conditions for 2-3 days at room temperature inside the room. After desirable fermentation, fermented doughs are then sun dried for 2-3 days to obtain dry starters which are exclusively used to ferment cereals into mild/strong alcoholic beverages (Tamang 2010a; Anupma et al. 2018).Ethnic starters have different vernacular names such as *marcha* in India and Nepal, *ragi* in Indonesia, *bubod* in Philippines, *chiu/chu* in China and Taiwan, *loogpang* in Thailand, *nuruk* in Korea, and *men* in Vietnam (Tamang et al. 1996, 2010a; Dung et al. 2007; Jung et al. 2012; He et al. 2019), which are used as starters for a number of fermentations based on rice and cassava or other cereals in Asia. There are several major types of ethnic amylolytic mixed starters in dry and ball-flatted discs shaped sold in local markets in India, Nepal, Bhutan, China, Thailand, Myanmar, Cambodia, Laos, Malaysia, Indonesia, Korea, Japan, Singapore, Taiwan, etc. (Table C-D). Calmette (1892) was the first to report the presence of several wild yeast species accompanied by *Amylomyces, Mucor, Aspergillus* and 30 different bacteria in starters used in China.

Table C: Amylolytic starter cultures of Asia and their alcoholic products.					
Country	**Dry Starter**	**Substrate used**	**Alcoholic beverage**	**Filamentous moulds**	**References**
Bhutan	*Marcha, Phab or pho* or *chang*	Maize	*Ara*	Unknown	Uchimura et al (1990); Tamang (2016c); Pradhan and Tamang (2019)
China	*Chiu-yueh*	Rice, wild herbs	*Lao-chao/ Tien-chiu-niang*	*Rhizopus, Amylomyces, Hansenula, Torulopsis*	Hesseltine et al. (1988); (Lee and Lee 2002); Tamang et al. (2012)
	Daqu	Sorghum	*Fen*	*Paecilomyces variotii*, *Aspergillus oryzae* and *Aspergillus terreus*	Zheng et al. (2011); Zheng et al. (2015)
	Hong-qu/yao-qu	Red rice	*Mijiu*	*Rhizopus oryzae*, *R. microsporus* and *Aspergillus* sp.	Lv et al. (2012a-b); Lv et al. (2013); (Park et al. 2016)
	Phab	Finger millet/barley	*Chyang/Chee* (Distilled)/ *Aarak*	Unknown	Tamang et al. (2012)
Cambodia	*Medombae*	Rice, Spices, herbs, and a sweetener are ingredients	*Sombai*	*Mucor* sp. and *Rhizopus oryzae*	Chim et al. (2015); Yamamoto (2016); Chay et al. (2017)
Indonesia	*Ragi*	Rice	*Tape, Berm*	*Amylomyces rouxii, Mucor indicus, Hansenula* sp. *Rhizopus oligosporus,*	Hesseltine and Ray (1988); Hesseltine et al. (1988a); Ohba et al. (1989)
Japan	*Koji*	Rice rice, or sometimes steamed legume beans	*Miso, saké, shoyu, shochu*	*Aspergillus oryzae* and *Rhizopus jawanicus*	Suganuma et al. (2007); Tamang (2010b); Bokulich et al. (2014); Akasaka et al. (2018); Uchida et al. (2019)
Korea	*Nuruk*	Rice, herbs or wheat flour	*Makgeolli, takju, Ewhaju, sojo, yakju*	*Aspergillus oryzae*, *A. niger, Lichtheimia Corymbifera, L. ramosa, Rhizopus oryzae, R. microspores, Rhizomucor. Pusillus, R. variabilis), Mucor racemosus* and *Syncephalastrum racemosum*	Yang et al. (2011); Jung et al. (2012); Shin et al. (2017)

Table C: Amylolytic starter cultures of Asia and their alcoholic products.

Country	Dry Starter	Substrate used	Alcoholic beverage	Filamentous moulds	References
Malaysia	*Tapai*	Rice, wild herbs	*Juipaing*	*Amylomyces rouxii* and *Rhizopus* sp.	Dung (2004)
Nepal	*Marcha*	Rice, plant herbs	*Jao ko jaanr* (barley), *Gaboon*	Unknown	Tamang et al. (2016a)
	Mana	Wheat and herbs	*Kodo ko jaanr*	*Aspergillus oryzae* and *Aspergillus flavus*.	Nikkuni et al. (1996); Shrestha and Rati (2002); Hui et al. (2012)
	Manapu	Rice, *manawasha* (white flower of a wild plant), and black pepper	*Poko*	*Rhizopus* sp. and *P. pentosaceus*	Hui et al. (2012); Tamang et al. 2016a
Philippines	*Bubod*	Rice, wild herbs ginger powder	*Basi/binubadan*	*Mucor circinelloides*, *M. grisecyanus*, *Rhizopus cohnii*	Hesseltine and Kurtzman (1990); Elegado (2016)
Thailand	*loogpang*	Rice and wild herbs	*Krachae* or *nam-khaao* or *sato*	*Amylomyces* sp., *Aspergillus* sp., *Mucor* sp., *Penicillium* sp. and *Rhizopus* sp.	Tanimura et al. 1977; Limtong et al. (2002); Khapudang et al. (2018); Kristbergsson and Otles (2016); Daroonpunt et al. (2016)
Vietnam	*Benh men* or *Men*	Rice, wild herbs, spices	*Ruou nep*	*Absidia corymbifera, Amylomyces rouxii, Botryo basidiumsubcoronatum, Mucor circinilloides, Mucor indicus Rhizopus oryzae, Rhi. microsporus, Xeromyces bisporus*	Lee and Fujio (1999); Dung et al. (2006); Dung et al. (2007); Thanh (2008)

Table D: Amylolytic starter cultures of India and their alcoholic products.

States	Ethnic communities	Amylolytic starter culture	Substrate used	Local name (Liquor)	Filamentous moulds	References
Arunachal Pradesh	*Apatani*	*Phut, epo*	Rice	*Chu*	Unknown	Shrivastava et al. (2012)
	Deuri and *khampti*	*Si-ye*	Glutinous rice, old starter, leaves of the plant *Leucas aspera* Spreng (local name *Zola*)	*Opo*	Unknown	Shrivastava et al. (2012)
	Nocte	*Pee/ Bichhi*	Rice, old starter, and *Piper betle* Linn	*Jumin*	Unknown	Bhatt et al. (2018)
	Thangsa/ Singpho/ Sulung/ Hill miri/ Tagin/ Wancho/ Sherdukpen	*Ipoh/ Chho/ Epope/ Bokha/ Phab/ Epchi/ Paa*	Rice, old starter, leaves of *Scoparia dulci*s Linn. (*Phansim)* and *Leucas lanata* Benth. (*Khamo*)	*Apong*	Unknown	Tiwari and Mahanta (2007); Shrivastava et al. (2012); (2012a); Ray et al. (2016)
	Mishmi and *Adi*	*Pee*	Rice, old starter, and tender leaves of *Artocarpus lakoocha* Roxb and *Mangifera indica* Linn.	*Opo*	Unknown	Shrivastava et al. (2012); Khapudang et al. (2018)
	Monpa	*Pham*	Rice, old starter, and tender leaves of *Solanum khasianum* Linn. (*Ichosu*)	*Themsing Kongpu* (Finger millet), *Baang* - Chang (Barley)	Unknown	Shrivastava et al. (2012a); Pandey et al. (2017)
Assam	*Dimasa*	*Humao*	Rice, old starter, and tender leaves of *Clerodendrum indicum* Linn. and fresh leaves of *Cissampelos pariera* Linn.	*Judima*	Unknown	Chakrabarty et al. (2014)
	Ahom	*Xaj-Pitha*	Rice and herbs	*Xaj-pani*	Unknown	Bora et al. (2016)
	Deori	*Mod Pitha*	Rice and Plant material (huge number 30)	*Sujen*	Unknown	Deori et al. (2007)
		VekurPitha	Rice and Plant material	*Ahom*	Unknown	Saikia et al. (2007)
	Adivasi	*Dabai*	Rice	*Haria*	Unknown	
	Karbi	*Thap*	Rice	*Arak*	Unknown	
Assam	*Garo*	*wansi*	Rice	*Chu*	Unknown	Narzary et al. (2016)
	Mishing	*Apong kusure*	Rice	*Apong*	Unknown	

	Bodo	*Amou/Perok-khushi*	Glutinaceous rice (*mwibra*) and wild herbs (*Ananascomosus L., Musa balbisiana, Arthocarpusheterophyllus, Scopariadulcis,*	*Jou*	*Amylomyces rouxii*, *Fusarium oxysporum* and *Rhizopus oryzae*	Das et al. (2017)
Himachal Pradesh		*Keem/ Phab and dheli*	Barley *Cannabis sativa, Sapindus mukorossi*	*chhang, jau chhang and sura*	Unknown	Thakur and Bhalla (2004)
Meghalaya	*Pnar, Jaintia*	*Thiat*	Rice, *Amomum aromaticum* Roxb (*khawiang* leaves).	*Kiad,* *Chubitchi* *Wanti*	Unknown	Samati and Begum (2007); Mishra et al. (2018; 2019)
Manipur	*Meithei*	*Hamei*	Rice and wild herbs *Albizi amyriophylla* (yangli)	*Atingba, Yu* (distilled liquior) *Chameli*	*Mucor* sp. and *Rhizopus* sp	Tamang et al. (2007); Jeyaram et al. (2009); Nath et al. (2019)
Mizoram	*Mizo*	*Dawidim/ Chawl*	Rice, wild herbs	*zupui, zufâng, tin-zû*	Unknown	Tamang (2020)
Nagaland	*Angami*	*Khekhrii*	Germinated rice	*Zutho/ Zhuchu, Peyazu*	*Rhizopus* sp.	Teramoto et al. (2002); Jamir and Rao (1990); Jamir and Deb (2014)
	Khasi	*Yei*	Germinated rice	*U Phandieng*	Not reported	
Sikkim	*Nepali*	*Marcha*	Rice, old starter culture, *Plumbago zeylanica* L. (guliyojara), *Buddleja asiatica* Lour (bheem-senpaate), sengreknna' flowers, ginger and red dry chilli	*Bhaati jaanr* (fermented rice beverages), Makai ko jaanr (fermented maize)	*Aspergillus oryzae, Mucor circinelloides Rhizopus chinensis. Rhizopus oryzae, Mucor praini* and *Absidia lichtheimi*	Tamang and Sarkar (1995); Tamang et al. (1996); Thapa and Tamang (2004); Tsuyoshi et al. (2005)
Tripura	*Jamatia/ Kalai*	*Chowan*	Rice and herbs	*Chuwak*	Not reported	Ghosh et al. (2016); Tamang (2020)
Uttarakhand	*Bhotiya*	*Balma*	Roasted wheat flour and spices	*Chhang*, *Jaan*	*Rhizopus* and *Aspergillus*	Bhardwaj et al. (2016); Das and Pandey (2007); Roy et al. (2004).
Uttarakhand	*Jaunsari*	*Keem*	Wheat, plants	*Soor*	Unknown	Rana et al. (2004)
West Bengal	*Santhal, Bonda,*	*Bakhar/ Ranu*	Rice and plant parts	*Haria* and *Pachwi*	*Amylomyces* sp., *Mucor* sp. and *Rhizopus*	Das et al. (2012a)

Filamentous moulds enzyme activities

Filamentous moulds are the preferred source of best industrial enzymes because of their abilities to secrete excellent capacity for extracellular enzymes (Jun 2011). *Aspergillus* species are source of 25% of all industrial enzymes, the reason behind that fungi are heterotrophic organisms (Østergaard and Olsen 2011). It is able to utilize extracellular sources of organic energy and material for, growth and reproduction (Park et al. 2017). The fungi can only absorb small molecules through their cell walls and an enzymatic digestion outside the mycelium is therefore often required (Hofrichter 2010). The fungi secrete a complex system of enzymes required for the digestion to the insoluble materials. *Aspergillus niger*, *Aspergillus oryzae* and *Trichoderma reesei* are most important production organisms in industrial fermentations applications (Frisvad et al. 2018). Xu et al. (2017) reported that during *daqu* preparation method the microorganism grow and produce several enzymes, such as amylase, cellulase, esterase, lipase and protease.

Amylase is starch hydrolyzing enzyme, that hydrolyze starch molecule into sugar (Saranraj and Stella 2013; Omemu et al. 2015), which act on internal alpha 1-4 glycosidic bonds of amylose and amylopectin polymers (Wanderley et al. 2017; Sunitha et al. 2012). The development of amylases began in 1811, when Kirchhoff discovered the first starch degrading enzyme (Gupta et al. 2003; Kumar and Chakravarty 2018; and Martin et al. 2019). They are present in all types of life, such as bacteria (Padhiar and Kommu 2016; Fentahun and Kumari 2017); fungi (Alves et al. 2002; Khokhar et al. 2011; Irfan et al. 2012; Grover et al. 2013); yeast (Yaicin and Corbaci 2013; Carrasco et al. 2016; and Thongekkae and Kongsanthia 2016). Among the various microorganisms that are employed for amylase production, fungi are the most reliable because of their more acceptable GRAS (Generally Regarded As Safe), grow like a

hyphal mode, good tolerance level to water activity (a_w), and high osmotic pressure condition (Singh et al. 2014). It has also the advantage of being secreted extracellular amylases in the brewing industry for decades (Gopinath et al. 2017). Amylase was first reported in 1894 by the Japanese scientist Jokichi Takamine at Peoria, Illinois (USA) from a fungal source and was used as a pharmaceutical aid for the treatment of digestive disorders (Akatın 2019; Saranraj and Stella 2013). Filamentous moulds are well known for production of α-, β-, and γ-amylases but α-amylase is well, being the most dominant (Gopinath et al. 2017). Fungal amylases are commonly used to make oriental foods (Saxena et al. 2015). In amylolytic starter culture *xaz-pitha* of Assam have existence of amylase producers notably *Aspergillus* sp., *Mucor circinelloides* and *Rhizopus delemar* (Bora et al. 2016). Kim et al. (2011) found that the Korean starter culture called *nuruk* has α-amylase producing fungi such as *Aspergillus flavus, Aspergillus oryzae, Lichtheimia* sp. and *Rhizopus oryzae* strains (Kim et al. 2017).

Cellulose ($C_6H_{10}O_5$) is the most ubiquitous organic biopolymer on earth (Zhang and Zhang 2013; Gupta et al. 2019). It is composed of homopolymer of linear chain of glucose, monomers links of β (1→4) linked D-glucose units (Kumar and Chakravarty 2018). Naturally, the degradation of cellulose is regulated by an enzymatic system called cellulases (Ahmed and Bibi 2018). Cellulase are the enzymes that hydrolyze enzyme β -1,4 linkages in cellulose chains (Imran et al. 2019). Cellulase are synthesized by a large type of organisms including fungi, bacteria, protozoans, plants and animals (Kuhad 2011; Srivastava et al. 2018). The enzymatic hydrolysis of cellulose is mediated by a combination of three major types of cellulases i.e. endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) (Sajith et al. 2016). Cellulases are increasingly being used in a large variety of industrial and approximate 20% overall market of enzyme around the world (Srivastava et al. 2018). It is used in

the textile, laundry, pulp, biofuel, paper, feed and food industry, as well as an additive in detergents and for improving digestibility of animal feeds. Fungi are the most studied organisms due to their abilities to produce large amounts of cellulases and hemicellulases, which are secreted into the medium and are subsequently easily extracted and purified (Ahmed and Bibi 2018). *Aspergillus niger* secretes large amounts of cellulolytic enzymes that have optimal activities for efficient hydrolysis of lignocellulosic biomass (Godoy et al. 2018).

L-asparaginase (L-asparagine amidohydrolase, E.C.3.5.1.1) is a tetrameric hydrolytic enzyme that catalyzes the deamination of L-asparagine into L-aspartic acid and ammonia (Cachumba et al. 2016; Souza et al. 2017).The substrate L-asparagine is on-essential and neutral amino acid which is required for the development and growth of tumour cells (Sanjotha 2017). However, L-asparagine is synthesized within a cell with the support of asparagine synthetase in normal cells. So, the cancer cells differ from normal cells in decreased expression of L-asparagine. L- Asparaginase has received considerable attention for the last few decades due to its applications in the fields of medicine, pharmaceuticals and food industry. In food industry, it is used to prevent the acrylamide formation when foods are processed in high temperature. L-asparaginase was found in various species, including animals, plants, fungi, bacteria and archaea (da Cunha et al. 2019).

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are generally hydrolytic enzymes which catalyses the hydrolysis and synthesis of long-chain acylglycerols (Dellamora-oritz et el. 1997). They catalyse the hydrolysis triglycerides into triacylglycerol to glycerol and fatty acid at the lipid water interface and also catalyse reverse reaction such as esterification and interesterification. This type of reaction is called lipolytic reaction (Dellamora-oritz et el. 1997). Lipase is found in plants (Bhardwaj et al. 2001),

animals (Carriere et al. 1994) as well as in microorganisms (Olempska-Beer et al. 2006; Mehta et al. 2017). Depending on substrate specificity lipases are divided into three groups: (a) Non-specific lipase: *Candida rugosa, Chrobacterium viscosum, Staphylococcus aureus* and *Thermomyces lanuginosus*), (b) 1-3, specific lipase or region-specific lipase: *Aspergillus niger*, *Penicillium roquefortii, Rhizopus delemar* and *Rhizopus oryzae*, produce region-specific lipase, (c) Fatty acid specific lipase: *Geotrichum candidum* (Ribeiro et al. 2011). Lipase producing microorganisms first described by Eijikmann (1901). Lipase producing microorganisms mainly isolated from soil, spoiled food, pickle, oil seeds and milk (Nwuche and Ogbonna 2011; Mehta et al. 2017). Lipases are isolated from bacteria, fungi, actinomycetes and yeast eg., *Chromobacter, Pseudomonas, Staphylococcus, Candida, Aspergillus, Mucor, Rhizopuus,* and *Burkholderia* are commonly utilised for lipase production (Sharma et al. 2011; Furini et al. 2018).

Proteases are ubiquitous entity found throughout the universe, mainly in plants, animals and microbes (Banerjee and Ray 2017; Razzaq et al. 2019). Proteases are a group of hydrolytic enzymes which catalyse the hydrolysis of peptide bonds present in protein and polypeptide chains of amino acid and split them into smaller polypeptides or else free amino acids (Souza et al. 2015; Razzaq et al. 2019; Sharma et al. 2019). During the period of 2014-2019 the global demand of protease enzyme has been growing compound annual with a growth rate of 5.3% (Banerjee and Ray 2017; Raveendran et al. 2018). They are widely used in commercial applications in various industries such as food, pharmaceutical, detergent, and leather (Singh et al. 2016; Razzaq et al. 2019). On the basis of catalytic activities and nature of reactive group in the catalytic site, proteases are classified (Razzaq et al. 2019). Two different type of protease found i.e. endopeptidase and exopeptidase (Mamo and Assefa 2018). Also, proteases are

classified into three groups, that is, neutral, acidic and alkaline proteases based on their acid-base natures (Tavano et al. 2018). One another basis catalytic action, protease has been grouped into four categories as aspartic protease, cysteine protease, metalloprotease, and serine proteases (Mamo and Assefa 2018). At their active sites, cysteine proteases contain cysteine residues and are mostly produced by fungal species such as *Aspergillus oryzae* and *Sporotrichum pulverulentum* (Banerjee and Ray 2017). Fungal proteases are among the hydrolytic enzymes most essential and extensively studied (Banerjee and Ray 2017). Fungal protease has attracted the attention because fungi can produce extracellular protease which is easily separated from mycelium. Also, it can grow on low cost substrates and secretes high amount of protease (Souza et al. 2015). Fungi are the main sources of acid proteases and they work better at pH range of 2.0-5.0. *Aspergilli* is the most dominant group for protease production in filamentous moulds (Tavano et al. 2018). Another common producer for protease is *Mucor,* Pen*icillium, Rhizopus* and *Neurospora* (Alves et al. 2002; Sharma et al. 2015). Xylan is second most abundant principle type of the hemicelluloses (Figueiredo et al. 2019). It is a linear backbone of β-1,4-linked d-xylopyranose residues (Walia et al. 2017; Collins et al. 2005; Polizeli et al. 2005; Bhardwaj et al. 2019). Xylanolytic enzymes are essential to catalyse the hydrolysis of complex xylan structure (Collins et al. 2005; Sakthiselvan et al. 2014). Xylanases (EC 3.2.1.8) hydrolyze xylan fibre to shorter sugar residues which have wide applications in industry (Goulart et al. 2005). Xylanases have great potential for industrial applications in the bioconversion of lignocelluloses into sugar, ethanol and other useful substances, feed, paper, in the clarification of juices and wines, in the improvement of the nutritional quality of silage and green feed as well as in the removal of waste paper processes (Patel and Savanth 2015; Shabeena et al. 2017). Xylanases are produced by diverse group of organisms

such as bacteria, algae, fungi, actinomycetes, protozoa, and arthropods (Bhardwaj et al. 2019; Hunt et al. 2016; Collins et al. 2005). Filamentous fungi are particularly interesting among microbial sources, because they secrete these enzymes into the medium and their levels of xylanase are much higher than those found in yeasts and bacteria (Cunha et al. 2018). Recently, interest in xylanases have markedly increased due to their potential applications in the food and beverage industries, feedstock improvement and the quality improvement of lignocellulosic residues (Sakthiselvan et al. 2014). Xylanases are produced on an industrial scale mainly by *Aspergillus* and *Trichoderma* (Bhardwaj et al. 2019).

Antinutritive degrading factor activities

Most foods consumed by humans are based on cereals that contain high amounts of anti-nutritional factors (Samtiya et al. 2020). Starter culture is prepared with cereals and plants herbs which are used for indigenous alcoholic beverages preparation (Das et al. 2012a). During the preparation of starter culture at the time of fermentation is thought to reduce the anti-nutrient (Lecithin, Phytates, saponins, tannins, and polyphenols) content in cereal grains significantly (Asres et al. 2018; Nkhata et al. 2018). During the fermentation process, it activates various endogenous enzymes and results in product with reduced anti-nutritional factors (Greiner and Konietzny 2006a; Samtiya et al. 2020). There are also significant amounts of anti-nutrients, disaccharides and oligosaccharides in cereals and legumes which are also used as weaning food (Adeyemo and Onilude 2013). Starter culture contains also other natural toxicants including tannins, phytoacid, protease and trypsin inhibitors, saponins, metal chelates, cyanogens, isoflavonoids, phytoalexins, flatus factors, etc (Adeyemo and Onilude 2013). Microorganisms present in these starter cultures play an important role in

degradation of various anti-nutritional factors, resulting an increase the nutritional value (Nkhata et al. 2018). Filamentous moulds are good sources of antinutritive degrading factors (Nkhata et al. 2018).

Laccase (E.C. 1.10.3.2, p-benzenediol: oxygen oxidoreductases) is a copper-containing an oxidoreductase able to catalyze the oxidation of various aromatic compounds (particularly phenolic compound) with the reduction of oxygen to water (Lu and Miyakoshi 2015; Agrawal et al. 2018). Laccase converts molecular oxygen to water and oxidizes substrates into free radicals (diphenols, methoxy-substituted monophenols, aromatic and aliphatic amines). Laccase was first observed by Yoshida in 1883 in the sap of *Rhus vernicifera*, a Japanese lacquer tree. Laccase enzymes have been predominately present in fungi, bacteria, plants, and insects (Agrawal et al. 2018). First fungal laccase was reported by Arregui et al. (2019). As laccase producers, a large number of fungi have been confirmed, with the most recognized white rot fungi. Among ascomycetes, deuteromycetes and basidiomycetes, white rot basidiomycetes were widely studied for the development and characterization of laccase (Couto et al. 2006).

Phytase (myo-inositol hexakisphosphate phosphohydrolase) catalyzes the stepwise removal of phosphate moieties from phytic acid (myo-inositol hexakisphosphate), thereby generating myo-inositol and inorganic phosphate (Ahmad et al. 2018). The first Phytases was reported in 1907 by Suzuki et al. in the path of rice bran hydrolysing studies. Phytate is the storage form of phosphate in nature, and mostly present in cereals, nuts, legumes, and oil seeds (Singh et al. 2013a). Phytate are potentially chelate multivalent cations (Such as Zn^{2+}, Mg^{2+}, Ca^{2+} and Fe^{2+}) and minerals so that able to reduce their bio-availabilities and nutritive qualities of food (El-Batal and Karem. 2001). Also, animal excreted phytic acid is degraded by microorganisms in the soil and the phosphorus released at high concentrations enters the rivers where it induces

eutrophication (Gargova et al. 1997). Phytase is widespread in plant, animals and microorganisms (Wyss et al. 1999; Lee et al. 2005; Farias et al. 2018). Fungal phytase was first reported by Shieh and ware in 1968. *Aspergillus ficuum* NRRL 3135 was most efficient source of phytase due to its GRAS nature and also due to its excessive secretory potential (Jatuwong et al. 2020). Phytase-catalysed dephosphorylation of naturally present iron phytate chelates in whole grain products is a potential option for increasing bioavailability of iron in the diet (Lee et al. 2005). Therefore, phytase is considered to be potential candidate for use as an enzyme that have great value in enhancing the nutritional quality of phytate – rich food products (Afinha et al. 2010; Jatuwong et al. 2020). WHO (2012) report suggested that 3-phytase from *Aspergillus niger* used as a food for humans and it found to safe for consumption. Phytase is classified according to its catalytic mechanism, pH optima (acid or alkaline phytases) and site of phytate hydrolysis initiation (Greiner and Konietzny 2006a; Irshadet al. 2017). Therefore, phytases are measured to be potential applicant for use as an enzyme that have great value in enhancing the nutritional quality of phytate-rich foods and feeds (Afinah et al. 2010).

Tannins are reported as the fourth most abundant group of compounds which are derived from secondary metabolites in plant parts like leaves, barks (Brahmbhatt et al. 2014; Dipak and Sheela 2015; Godoy et al. 2018). Tannins are water-soluble phenolic compounds, and they differ from most other phenolic compounds in their unique abilities to precipitate protein and other macromolecules in solution (Adamczyk et al. 2017; Banerjee and Mahapatra 2012). The tannins are divided into two groups: hydrolyzable tannins and condensed tannins. Condensed tannins are more resistant to microbial degradation than hydrolyzable tannins and can be soluble in aqueous organic solvents, depending on their structures (Girdhari and Peshwe 2015; Naumann et al.

2017). Tannin acyl hydrolase (EC 3.1.1.20), known as tannase, catalyzes the hydrolysis of esters and depside bonds of hydrolysable tannins, such as tannic acid, and esters of gallic acid, producing glucose and gallic acid (Lima et al. 2014; Dipak and Sheela 2015). Tannase has wide range of industrial application in different food and feed, beverages, pharmaceuticals, cosmetics, chemicals and brewing industries, in preparation of gallic acid, in instant tea, coffee flavored soft drinks, clarification of fruit juices and beer (Beniwal et al. 2013). Tannase is extracellular inducible enzyme that can be produced by fungi, bacteria and plants (Brahmbhatt et al. 2014). Filamentous moulds are most dominant tannase producers among all microbial communities such as filamentous moulds genera *Aspergillus* and *Penicillium* (Lima et al. 2014). Tannins are associated with antinutritional effects due to the development of protein complexes, where they induce a decrease in nutrient absorption and metal ions, amino acids and polysaccharides (Godoy et al. 2018; Molino et al. 2019).

Table: E. Detail of fungal extracellular enzyme applications in diverse field.

Enzymes	Genera/ Species	Application	Reference
Amylase	*Aspergillus flavus, Mucor sp.*	Baking, brewing, Starch and glycogen hydrolysis, Clarification of juice, Starch liquefaction and saccharification, increasing shelf life and improving quality by retaining moist, elastic and soft nature, Bread softness and volume, flour adjustment, ensuring uniform yeast fermentation, Juice treatment, low calorie beer.	Souza 2010; Al-Maqtari et al. (2019)
Cellulase	*Aspergillus niger*	Cellulose hydrolysis, Clarification of fruit juice	Villena and Gutiérrez-Correa (2006); Ja'afaru (2013)
L- Asparaginase	*Aspergillus aculeatus, Aspergillus nidulans, Aspergillus niger, Aspergillus sydowii, Cladosporium* sp., *Fusarium roseum Mucor hiemalis, Penicillium* sp. *Trichoderma viride*	Prevent the formation of acrylamide when foods are processed in high temperatures,	Cachumba et al. (2016); Abdelrazek et al. (2019)
Lipase	*Aspergillus ibericu, Aspergillus niger, A. oryzae, Aspergillus versicolor, Rhizomucor variabilis, Rhizopus oryzae, Thermomyces lanuginosus*, *Penicillium* sp.	Flavour development in dairy products, Cheese flavour, in-situ emulsification for dough conditioning, support for lipid digestion in young animals, synthesis of aromatic molecules.	Geoffry and Achur (2018); Drozłowska (2019)

Protease	*Aspergillus niger Aspergillus parasiticus, Aspergillus usamii, Aspergillus flavus, Aspergillus niger, Aspergillus oryzae, Penicillium chrysogenum, Mucor circinelloides*, *Rhizopus*	Brewing Meat tenderization Coagulation of milk Bread quality improvement, Protein hydrolysis, milk clotting, low-allergenic infant food formulation, enhanced digestibility and utilization, flavour improvement in milk and cheese, meat tenderizer, prevention of chill haze formation in brewing.	Souza et al. (2015); Sharma et al. (2019)
Xylanase	*Aspergillus* sp., *Fusarium* sp., *Penicillium chrysogenum, Penicillium citrinum*	Clarification of fruit juice, Beer quality improvement	Okafor et al. (2007); Ja’afaru (2013); Wadia and Jain (2017); Walia et al. (2017)
Phytase	*Penicillium polonicum*	Release of phosphate from phytate, enhanced digestibility.	Ahmad et al. (2018); Lei et al. (2013)
Laccase	*Botrytis cinerea, Penicillium chrysogenum, Penicillium oxalicum, Trametes hirsuta Trametes villosa*	chemical synthesis, bio-bleaching of paper pulp, bioremediation, biosensing, wine stabilization and textile finishing, Clarification of juices, flavour enhancer (beer)	Jian-rong and Dang (2006), Raveendran et al. (2018); Senthivelan et al. (2019)
Tannase	*Aspergillus niger, Cladosporium parahalotolerans, Paecilomyces variotii, Trichoderma harzianum*	Eliminating the bitter taste of fruit juices and tea infusions by enzymatic treatment improves the consistency of these drinks	Al-Mraai et al. (2019); Kumar et al. (2019a)

MATERIAL AND METHODS

MEDIA USED

1. Cellulase assay medium

a. Yeast Extract Peptone Agar

b. Carboxy-methylcellulose (CMC) 0.5%

2. Czapek-Dox broth medium

a. Glucose	2.0 g
b. L-asparagine	10.0 g
c. K_2HPO_4	1.52 g
d. Potassium chloride	0.52 g
e. Magnesium sulphate	0.52 g
f. Cupric nitrate	0.001 g
g. Zinc sulphate	0.001 g
h. Ferrous sulphate	0.001 g
i. Phenol red	0.009 g
j. Distilled water	1.0 L
k. pH	7.2 ± 0.2

3. Czapek Dox minimal medium (for tannase)

a. Tannic acid	10.00 g
b. D-Glucose	10.00 g
c. $NaNO_3$	6.00 g
d. NH_4Cl	1.0 g
e. KH_2PO_4	1.52 g
f. K_2HPO_4	0.50 g
g, KCl	0.52 g
h. $MgSO_4.7H_2O$	0.52 g

i. $CaCl_2$	0.01 g
j. $Cu(NO_3)_2.3H_2O$	trace
k. $FeSO_4.7H_2O$	trace
l. $ZnSO_4.7H_2O$	trace
m. Distilled water	1.0 L
l. pH	5.0±0.2

4. Gelatin Agar Medium (For Protease Test)

a. Gelatin	20.0 g
b. Caesin	10.0 g
c. Sodium Chloride	10.0 g
d. Sodium taurocholate	5.0 g
e. Sodium bicarbonate	1.0 g
f. Agar	15.0 g
g. Distilled water	1.0 L
h. pH	8.5 ± 0.2

5. L- Asparaginase Test Medium (Modified Czapex Dox Medium)

a. Glucose	2.0 g
b. L-asparagine	10.0 g
c. K_2HPO_4	1.52 g
d. Potassium chloride	0.52 g
e. Magnesium sulphate	0.52 g
f. Cupric nitrate	0.001 g
g. Zinc sulphate	0.001 g
h. Ferrous sulphate	0.001 g
i. Phenol red	0.009 g

j. Agar	20.0 g
k. Distilled water	1.0 L
l. pH	7.2 ± 0.2
6. Malt-Extract Agar	(M137, HiMedia, Mumbai)
a. Malt Extract agar	50.0 g
b. Distilled water	1 L
7. Modified Czapek dox agar	(M075, HiMedia, Mumbai)
8. Physiological Saline (0.85%)	(Andrew 1992)
a. Sodium Chloride	0.85 g
b. Distilled water	100 ml
9. Potato Dextrose Agar (PDA)	(M096, HiMedia, Mumbai)
a. Potato dextrose agar	39.0 g
b. Distilled water	1 L
10. Potato Dextrose Broth (PDB)	(M403, HiMedia, Mumbai)
a. Potato dextrose agar	39.0 g
b. Distilled water	1 L

11. Phytase screening medium (PSM) agar (Howson and Davis 1983)

a. sodium phytate	0.5%
b. NH_4NO_3	0.5%
c. KCl,	0.05%
d. $MgSO_4.7H_2O$	0.05%
e. $MnSO_4.4H_2O$	0.03%
f. $FeSO_4.7H_2O$	0.03%
g. Glucose	3%
h. Agar	2%

i. Distilled water 1.0 L

i. pH 5.5

12. Sabouraud Dextrose Agar (SDA) (SM063D, HiMedia, Mumbai)

13. Starch Agar (Gordonet al. 1973)

a. Starch 10% (w/v)

b. Trypyone 50 g

c. Yeast extract powder 15.0 g

d. Potassium dihydrogen phosphate 3.0 g

e. Agar 20.0 g

f. Distilled water 1 L

14. Xylanase test medium

a. Xylan 5.0 g

b. Peptone 5.0 g

c. Yeast Extract 5.0 g

d. K_2HPO_4 1.0 g

e. Magnesium sulphate 0.2 g

f. Agar 20.0 g

g. Distilled water 1.0 L

h. pH 7.0 ± 0.2

REAGENTS USED

1. Bovine serum albumin (BSA): BSA (2.0%) was prepared in citrate phosphate buffer (pH 5.0).

2. 0.2% Congo red solution

0.2 gm Congo red powder in 100 ml distilled water.

3. DNS Reagent

About 1 g of dinitrosalicylic acid (DNSA) (Merck, Germany) was dissolved in 50ml of distilled water. To this solution, 30 g of sodium tartarate tetrahydrate (Merck, Germany) was added in small lots, the solution turned yellow in colour. The 20 ml of 2N NaOH (Merck, Germany) was then added, which turned solution to transparent orange –yellow colour. The final volume was made to 100ml with the distilled water. The solution was stored in an amber coloured bottle (Salihu et al. 2015).

4. Phenolphthalein indicator

Weigh out 1.0 g of phenolphthalein. Prepare a 50% ethanol (ethyl alcohol) solution consisting of 50ml ethanol and 50 ml water. Dissolve the phenolphthalein thoroughly in the 50% ethanol solution.

5. 1% Starch solution

About 80cm^3 of distilled water was heated in a beaker. 1 gram of starch was weighed, mixed with a splash of distilled water. When the water was a near boiling, the starch was added to make up to 100cm^3 and brought to boil.

6. 1N Hydrochloric acid (HCl)*

One normal hydrochloric acid was prepared by adding 1.0 ml concentrated HCl to 10.0 ml of double distilled water.

7. 1N Sodium hydroxide (NaOH)*

One normal sodium hydroxide was prepared by dissolving 4.0 g of NaOH in 100 ml of double distilled water.

*These were used for adjusting the pH of the medium**.**

8. Lacto cotton blue

Dissolve 20 g of Phenol (solid), 20 ml of lactic acid and 40 ml of glycerol into 20 ml of distilled water (heat as gently as possible). Add 0.05 g of cotton blue, shake until

mixed well and filter until storage (Zhou and Li 2015). The wet mount preparation of lactophenol cotton blue (LPCB) is the most widely used method of staining and observing filamentous moulds. The preparation has three components: phenol that destroys all living organisms; lactic acid that retains fungal structures and cotton blue that stains the chitin in the cell walls of the fungi (Parija and Prabhakar 1995; Leck 1999).

9. Lugol's iodine/Iodine solution

Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	300 ml

Solution (10% potassium iodide and 5% iodine crystal)

10. Tannic acid (1.0%): The solution was prepared by dissolving 1.0 g of tannic acid in 100 ml of citrate-phosphate buffer of the desired pH.

11. Buffers

Buffers	**pH Range**	**Reagent**	**Method** (Sambrook and Russell 2001)
0.2 M Acetate buffer	5.0	Quantity (for 2L): Sodium acetate trihydrate 54.43 g Glacial acetic acid 12 ml Distil water 1988 ml	Combine the reagents and adjust the pH to 5.0 with 10 N NaOH
Citrate Phosphate	3.0–5.0	A: 0.1M solution of citric acid B: 0.2M solution of Na_2HPO_4	pH 3: 39.8 ml A + 10.2 ml B made up to 100 ml pH 4: 30.7 ml A + 19.3 ml B made up to 100 ml pH 5: 24.3 ml A + 25.7 ml B made up to 100 ml

CHEMICALS USED

1. Agarose	(MB094, HiMedia, Mumbai)
2. Ammonium acetate	(GRM295, HiMedia, Mumbai)
3. Ammonium molybdate	(GRM307, HiMedia, Mumbai)
4. Ammonium vanadate	(GRM478-100G, HiMedia, Mumbai)
5. Ammonium per Sulphate-APS	(MB003, HiMedia, Mumbai)
6. Beechwood	(MB141-10G, HiMedia, Mumbai)
7. Bovine serum albumin (BSA)	(MB083-5G, HiMedia, Mumbai)
8. Carboxy-methylcellulose (CMC)	(GRM329, HiMedia, Mumbai)
9. Chloroform	(MB109-500ml, HiMedia, Mumbai)
10. Copper (II) nitrate, $CuNO_3\ 3H_2O$	(GRM675-500G, HiMedia, Mumbai)
11. CTAB	(MB101-500, HiMedia, Mumbai)
12. Diethyl ether	(AS126-500ML HiMedia, Mumbai)
13. DNeasy Plant Mini Kit (50)	(69104 Qiagen)
14. Ethanol	(MB106, HiMedia, Mumbai)
15. EDTA	(GRM3915-100G, HiMedia, Mumbai)
16. ETBR	(RM813, HiMedia, Mumbai)
17. Gel loading dye	(G1881, Promega, US)
18. Go-taq green Master Mix	(M7122, Promega, US)
19. Guaiacol	(RM1118, HiMedia, Mumbai)
20. Iodine	(GRM1064-50G, HiMedia, Mumbai)
21. Isopropanol	(MB063-1L, HiMedia, Mumbai)
22. L-asparagine	(GRM041-25G, HiMedia, Mumbai)
23. ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′)	(ILS)
24. ITS4 (5′-TCCTCCGCTTATTGATATGC- 3′)	(ILS)

25. Mercaptoethanol (MB041-100ML, HiMedia, Mumbai)

26. Methyl red (I007, HiMedia, Mumbai)

27. Nessler's reagent (R010-1254ML, HiMedia, Mumbai)

28. Nuclease free Water (129115, Qiagen)

29. PEG (polyethylene glycol) (GRM3662-500G, HiMedia, Mumbai)

30. PCR- Gel Purification kit (A9281, ProMega, USA)

31. Potato Dextrose Agar (M096-500G, HiMedia, Mumbai)

32. Phenol: chloroform: isoamyl alcohol (25:24:1) (MB078-100ML, HiMedia, Mumbai)

33. Phenol red indicator (IO10-125ML, HiMedia, Mumbai)

34. Proteinase K (V3021, Promega, US)

35. Phenolphthalein (I009-125ML, HiMedia, Mumbai)

36. RNAse (A7973, Promega, US)

37. Sodium acetate (S2889, Merck, US)

38. Sodium Hydroxide Solution (MF8D, Merck Millipore, US).

39. Sodium phytate (GRM6226-25G HiMedia, Mumbai)

40. Sodium n-Dodecyl Sulfate (20% Solution w/v) (428018, Merck, US)

41. Starch Agar (M107-500G HiMedia, Mumbai)

42. Streptomycin (CMS220-5G HiMedia, Mumbai)

43. Tannic acid (GRM7541-500G HiMedia, Mumbai)

44. Trichloroacetic acid (TCA) (GRM6274-500G, HiMedia, Mumbai)

45. Tetra methyl ethylenediamine (5965-833, HiMedia, Mumbai)

46. Whatman No. 2 filter paper (WHA1001917, Whatman)

47. Xylan (MB141-10G, HiMedia, Mumbai)

48. 1×TAE buffer (ML016, HiMedia, Mumbai)

49. 10X TE Buffer. pH 8.0 (ML012-500ml, HiMedia, Mumbai)

50. 100-mM Tris–HCl (pH 8.0) (ML013-500ML, HiMedia, Mumbai)

51. 100 bp DNA ladder (MBT049-50LN, HiMedia, Mumbai)

52. 2 mercaptoethanol (MB041-100ML, HiMedia, Mumbai)

53. 3, 5- dinitrosalicylic acid (GRM-1582-25G, HiMedia, Mumbai)

54. 2-2’-Azino-bis- (RM9270-1G, HiMedia, Mumbai)

[3-ethyl benzthiazoline-6-sulfonic acid]

55. 200-mM Tris–HCl (pH 8.0) (ML013-100ML, HiMedia, Mumbai)

KITS USED

FASTDNA™-96 fungal/bacterial DNA kit (1196963001)

REFERENCE STRAINS OF FILAMENTOUS MOULDS

Certified fungal species and strains were being obtained for controls from National Collection of Industrial Micro-Organisms (NCIM) of **CSIR-National Chemical Laboratory, Pune.**

Sl. No.	Species	NCIM Reference No.
1	*Aspergillus niger*	1248
2	*Fusarium oxysporum*	1350
3	*Aspergillus niger*	1358
4	*Aspergillus oryzae*	1212
5	*Aspergillus flavus*	549

INSTRUMENT USED

1. ABI-DNA-Sequencer	(ABI 3500, HITACHI, Japan)
2. Analytical weighing balance	(Mettler, AX 204 Kolkata)
3. Autoclave	(Instrumentation India, Kolkata)
4. Biological Incubator	(Accumax, CIS-24BL, Kolkata)
5. Centrifuge	(Thermo Scientific, CL21, USA)
6. Compound Microscope	(Olympus, EX1000, Japan)
7. DE/Axio Imager A1 microscope	(Carl Zeiss, Oberkochen, Germany)
8. Desiccator	(DURAN, DIN-12491, USA)
9. Digital PH meter	(Orion 910003, Thermo Fisher Scientific, USA)
10. DNA kit	(Promega, USA)
11. Electrophoresis Unit	(Remi, R-24, Mumbai)
12. Gel doc Imaging System	(1000, Bio-Rad, 97-0186-02, USA)
13. High precision water bath	(Remi, RIME-1322, Mumbai)
14. Laminar Air Flow	(1386, Thermo Scientific, USA)
15. Magnetic stirrer	(Remi, 2MLH, Mumbai)
16. Mechanical oven	(Instrumentation India, Kolkata)
17. Microwave Oven	(Samsung, 28L Mumbai)
18. Moisture analyzer	(OHAUS/MB-45, USA)
19. Nano-DropND-1000	(Nano Drop Technologies, USA)
20. NGC Illumina-Miseq	(Illumina Platform, USA)
21. Orbital Shaker Incubator	(Remi, RSB-12, Mumbai)
22. Qubit Fluorimeter	(Invitrogen, Q33227, USA)
23. Phase contrast microscope	(Olympus, CKX41, Japan)/ DE/Axio Imager A1 microscope (Carl Zeiss, Oberkochen, Germany)
24. Spectrophotometer	(Eppendorf, Germany/ *Perkin-Elmer*, LAMBDA 950**,** USA))
25. Stomacher lab blender	(Seward, UK)

26.Thermal Cycler	(Applied biosystems-2720, USA)
27. UV light	(Gel doc 1000, Bio-Rad, 97-0186-02, USA)
28. UV-Transilluminator	(MD-25/HD-25, Wealtec, USA)
29. Vertical Laminar Air flow	(Thermo Scientific, 1386, USA)
30. Water bath Shaker	(Digilab, EX9UA, Mumbai)
31. Water Distillation unit	(Riviera, 72240020, Kolkata)
32. -20 Freezer	(Remi, ROFV-170, Mumbai)
33. -80 Freezer Vertical	(TSE240A, Thermo fisher, USA)

SOFTWARE USED

1. ChromasPro	(Technelysium-V1.34, Australia)
2. MEGA 7	(Pennsylvania State University Vl.7.0.26, USA)
3. PAST	(Palaeontological Association- V4.0, Norway)
4. QIIME	(University of Colorado- V2- 2019.10, USA)
5. Sequence Scanner	(Applied Biosystems-V1.0, USA)

METHODOLOGY

SURVEY

We conducted survey in the following sites for this Thesis: Basilakha, Gangtok and Pakyong of Sikkim; Shillong and Nongrem of Meghalaya; Kokrajhar, Jorhat, Moran and Sivsagar of Assam; Kangchup, Kakching and Phayeng of Manipur; Bangsul and Dharmanagar of Tripura; Banderdewa, Doimukh, Itanagar, Nirjuli and Pasighat of Arunachal Pradesh; Saitual of Mizoram and Kohima of Nagaland representing states of North East India. These places were chosen for survey and collection of traditional starter culture samples prepared by the ethnic communities, which were purchased/obtained during 2015-2017. Since the shelf-life of starter culture has been reported to be one year (Tamang 2010b), tentative date of starter culture production was confirmed from the seller so that old dry starters were not collected. General survey and documentation of indigenous methods of starter culture preparation was conducted in household using an questionnaire (Table F). The indigenous knowledge was documented for preservation of preparation method of traditional starter cultures.

SAMPLE COLLECTIONS

A total of 40 samples of traditionally prepared dry starters viz *marcha* (8 samples) from Sikkim, *thiat* (4 samples) from Meghalaya, *humao* (7 samples) of Assam, *hamei* (3 samples) of Manipur, *chowan* (4 samples) of Tripura, *phut* (6 samples) of Arunachal Pradesh, *dawdim* (3 samples) of Mizoram and *khekhrii* (5 samples) of Nagaland were collected directly from local markets and homes of the local producers in North East India in pre-sterile containers. Dry starter samples were transported to laboratory and stored in desiccators at room temperature since traditionally prepared dry starters have shelf life of more than one year.

Table F: Survey format for documentation of indigenous knowledge of dry starters preparation in North East India

I. General information **Date**:

1. Name of the Informant:
2. Ethnic group:
3. Name of:
   a. Village /Revenue:
   b. Sub-division:
   c. District:
   d. State/Province:
   e. Country:
4. Approximate number of house hold:
   a. House hold in village:
   b. Population of village:
5. Distance of the village from
   a. Nearest market (km):
   b. Nearest town (km):

II. Information on Amylolytic starter culture:

6. Name of dry starter:
7. Local name:
8. Ingredients:
9. Plant used:

Local Name of Plant	Botanical Name	Plant parts used	Ethnical values, if any

10. Flow sheet of traditional preparation of starter culture
11. Colour; appearance:
12. Time of storage:
13. Amount of old starter culture added:
14. Age of starter culture:
15. History:
16. Which type of drink are made from starter culture:
17. Which time sample prepare mostly:
18. Do you think that starter culture has medicinal values or play a role in promoting health?
19. Do you use starter culture for other purposes?
20. Are you economically dependent on these products? Yes/No
21. What is the approximate amount of monthly/annual production of these starter cultures? (ref: last month/year)
22. What is the approximate income from the sale of starter culture and products?

Remarks:

Name and signature of investigator:

ANALYSIS of MOISTURE CONTENT and pH

The moisture content of the samples was estimated by moisture analyser. The pH of homogenised samples was recorded by digital pH-meter.

MICROBIOLOGICAL ANALYSIS (FILAMENTOUS MOULDS)

Each sample of dry starter was taken from desiccator, then crushed coarsely by sterile spatula and 10 g of crushed powered sample was homogenised with 90 ml of 0.85% physiological saline in a stomacher lab blender 40 for 2 mins to make serial dilutions. 1 ml each of diluted samples (10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) was poured on malt extract agar and potato dextrose agar with addition of antibiotics (1% streptomycin) to suppress the growth of bacteria and then plates were incubated at 28ºC and observed for appearance of colonies till one week. The colonies appeared on plates were counted as colony forming unit (cfu/g). Colonies were selected on the basis of macroscopic and microscopic characteristics. Selected filamentous moulds were sub-cultured on new plates and purified and stores on slants at 4ºC for further studies.

MORPHOLOGICAL AND PHYSIOLOGICAL IDENTIFICATION

For each isolate, one- or three-point inoculations on petri plates containing approximately 25ml of media were applied. Fungal morphology was studied macroscopically by observing the colony features (surface color, reverse side color, shape and diameter) and microscopically by observation of fruiting bodies using stereomicroscope and the vegetative and asexual stages were observed by a DE/Axio Imager A1 microscope after staining freshly grown mycelia stained with cotton blue in MEA plates (Gaddeyya et al. 2012). Filamentous moulds were identified on the basis

of morphological features using the taxonomical keys described by Samson et al. (2004) Pit and Hocking (2009) and Watanabe (2010).

MOLECULAR IDENTIFICATION

Genomic DNA Extraction

The genomic DNA was extracted from mould cultures following the methods of Umesha et al. (2016). Mycelial mass from the culture plate was scraped out by sterile surgical blade and ground in sterile mortar pestle using 500 µL of extraction buffer [100-mM Tris–HCl (pH 8.0), 20-mM EDTA (pH 8.0), 1.4M NaCl, 2% CTAB and 0.2% 2 mercaptoethanol]. The mixture was transferred to fresh 1.5 ml tube with addition of 4-µL RNase, vortexed and incubated for 60 min at 37 °C and kept in a water bath for 60 min at 55°C. The 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the solution, mixed thoroughly for 5 min, then centrifuged at 14,000 rpm for 10 min. The aqueous clear phase was recovered and mixed with chloroform: isoamyl alcohol (24:1), centrifuged at 12,000 rpm for 5 min and the aqueous phase was recovered, then added 0.8 volume of cold 7.5 M ammonium acetate and 0.54 volume of ice-cold isopropanol and finally thoroughly mixed and kept at deep freezer for 12 h for precipitation of DNA. Solution was centrifuged at 14,000 rpm for 3 min and precipitated with absolute ethanol to recover DNA. The DNA was then rinsed twice with 1 ml of 70 % ethanol and resuspended in 100µL of 1X TE [200-mM Tris–HCl (pH 8.0), 20-mM EDTA (pH 8.0)] buffer for further use and stored at -20ºC. DNA quality was checked on agarose gel and concentrations were measured by using nanodrop spectrometer following the method described by Kumbhare et al. (2015).

PCR amplification

Polymerase chain reactions (PCR) of the internal transcribed spacer (ITS) region of filamentous moulds was amplified using the primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC- 3′) (Adekoya et al. 2017). The PCR reactions were performed in 25 μL of PCR pre-master mix solution (Promega, USA). The amplification steps were followed: initial denaturation at 94ºC for 5 min followed by 35 cycles consisting of 94ºC for 1 min, 54 C for 1 min, and 72ºC for 1 min, respectively; and final extension was made at 72ºC for 10 min in a Thermal Cycler. The PCR products were verified by electrophoresis on 1.0% agarose gel containing 0.7 mg/ml of ethidium bromide and visualized in UV light Gel doc. Approximate size of amplicons was determined using standard molecular markers.

Purification of the PCR amplicons

The amplified PCR products were purified using PEG (polyethylene glycol)-NaCl (sodium chloride) and precipitation solution (20% w/v of PEG, 2.5 M NaCl) with addition of 0.6 volumes of 20% PEG-NaCl to final volume of PCR products (Schmitz and Riesner 2006). The mixture was centrifuged at 12,000 rpm for 30 min, incubated at 37ºC for 30 mins, the aqueous solution was discarded and pellet was washed twice with 1 ml ice cold 70% freshly prepared ethanol (70%). The collected pellet was then air dried prior to elution in 20µl of nuclease-free water and finally the purified product was loaded in 1% agarose gel.

ITS Sequencing

The PCR-amplified products had been sequenced in a forward and reverse direction using ITS 1 primer (5’-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 primer (5′-TCCTCCGCTTATTGATATGC-3′) primer, respectively as per the method of Martin and Rygiewic (2005). The PCR reaction was carried out in 50 μL reaction volume containing 2.0 mM $MgCl_2$, 0.2 μM each primer, 0.2 mM dNTP, 0.5 mg [ml]-1 bovine serum albumin (BSA) and 0.04 U [μL]-1 tTaq DNA polymerase on a thermal cycler equipped with a heated lid. The thermal program included initial denaturation, enzyme activation at 95°C (6-10 min) followed by 35 cycles of complete the step [95°C for 1 min, 40°C for 2 mins and 72°C for 1 min and one cycle at 72°C for 10 min]. The amplified products were sequenced by an automated DNA Analyzer. These high-quality, double-stranded sequence data were analysed with the help of the BLASTn program and multiple sequence alignment.

Bioinformatics

The qualities of the raw sequences were checked by Sequence Scanner version 1.0 and were edited using software ChromasPro version 1.34. Sequences were compared with sequence entries in the GenBank of NCBI (National Center for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/Blast.cgi) using the Basic Local Alignment Search Tool for nucleotides (BLASTn) on the NCBI website (Pinto et al. 2012). For phylogenetic analysis, the available sequence of similar related organisms was retrieved in FASTA format and aligned using the clustal-W. Sequence alignment and phylogenetic tree were constructed using MEGA7.0 software by Neighbor-Joining methods using 1000- bootstrap replicates (Lutzoni et al. 2004).

TAXONOMICAL KEYS

Taxonomical keys for filamentous moulds isolated from dry starters of were illustrated based on macro- and micro-characteristics and ITS data. Macro-characteristics included colour and tint in colony surface and reverse, smell or fragrance, quantity of aerial hyphae, colony surface texture (cottony, powdery shrunken, sloppy, velvety, crustaceous, water soaked, embedded and yeast-like), colony margin (smooth, irregular, restricted, spreading), colony Pattern (arachnoid, radiate, flowery, and zonate), pigment exuded (colour, watery). Micro-characteristics included shape of hyphae (septate aseptate, clamp connection), spore (sporangiospores, chlamydospores and basidiospores) and size (length, width, thickness) (Hanlin 1999; Samson et al. 2004; Pit and Hocking 2009; Watanabe 2010; Kirk et al. 2013).

STATISTICAL ANALYSIS

Percentages of frequency and relative density of fungal species in samples were determined as per the method described by Doi et al. (2018). Frequency (%) was calculated by the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

Relative Density (%) was calculated by the equation:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all quadrats} \times 100}{\text{Total number of quadrats studied}}$$

Diversity indexes of filamentous moulds in samples were calculated by species richness (R), Shannon's diversity index (H), and species evenness (E) (Panda et al. 2010) using PAST (Paleontological STatistics) software version 3.26 (Hammer et al. 2001).

DATA AVAILABILITY

Sequences obtained were deposited at the GenBank-NCBI database with accessional numbers: MK396469-MK396484, MK396486-MK396500, MK778442- MK778449, MK796041-MK796045.

CULTURE INDEPENDENT TECHNIQUE (NEXT GENERATION SEQUENCING)

Genomic DNA Extraction, PCR amplification and Amplicon sequencing

Metagenomic DNA was extracted from samples of dry starters viz. *marcha* of Sikkim and *thiat* of Meghalaya using Promega DNA kit. For the amplification of fungal internal transcribed (ITS) regions, 50 ng genomic DNA was amplified for 26 cycles using KAPA HiFi HotStart PCR Kit (KAPA Biosystems Inc., Boston, USA) along with forward primer GCATCGATGAAGAACGCAGC and reverse primer TCCTCCGCTTATTGATATGC (Edduozi et al. 2013). Concentration of both the primers was kept at 0.5 μM each. The time and temperature profile for the PCR reaction was kept same as 16S rRNA amplification. The amplicons from first round PCR were analysed using 1.2% agarose gel. The 1 μl of diluted 1st round PCR amplicons were used for indexing PCR (2nd round). Here the amplicons from 1st round PCR were amplified for 10 cycles to add Illumina sequencing barcode adapters and the amplicons were normalized and pooled for Illumina MiSeq 2×300 bp sequencing.

Bioinformatics analysis

The raw sequences generated from MiSeq platform in high-throughput amplicon sequencing method were assembled using FLASH tool (Fast Length Adjustment of Short reads) and a paired end assembler for DNA sequences (Masella et al. 2012) The

assembled reads were subjected to quality filtering using via Quantitative Insights into Microbial Ecology (QIIME) 1.8 (Caporaso et al. 2010). Raw sequence reads were allocated by a closed reference-based OTU picking method to fungal operational taxonomic units (OTUs), using the UNITE reference databases. OTU picking was conducted using the 97% similarity threshold UCLUST approach (Edgar 2010). Taxonomic assignments were carried out using the naive Bayesian classifier of RDP (Wang et al. 2007). After rarefying all samples to the same sequencing depth (Blaalid et al. 2013) and (Bokulich et al. 2012), the alpha diversity indices such as Shannon, Shannon and Chao were calculated via QIIME pipeline.

Data Availability

The raw sequences obtained from the high-throughput sequencing effort were submitted to NCBI GenBank, available under the PRJNA376467 Bio-Project ID.

SCREENING OF EXTRACELLULAR ENZYME

Growth at Different Temperatures

Filamentous moulds were inoculated on MEA petri-dishes (diameter, 90 mm) containing 30 ml MEA and incubate at different temperature range 5°C, 10°C, 20°C, 30°C, 40°C, and 60°C, respectively. The mycelial diameter of the inoculated cultures was measured at 48 and 96 h (Serna-Jimenez et al. 2016).

Screening of extracellular amylase enzyme

Qualitative enzyme assay

Starch agar media was used for primary screening of the starch hydrolysis activity as per the method of Choi et al. (2005). Isolates were grown in Starch agar and were

incubated at 30°C. After the appreciable amount of the growth of mycelium, 1% iodine solution was added to the plates. Clear zone was observed for the organisms showing positive results (Choi et al. 2005). Diameters of the clear zones and fungal colonies were measured by millimeter ruler. The isolates showing positive zone in primary screening were subjected to quantitative screening method.

Quantitative enzyme assay

Production medium contained (g/l) $NaNO_3$- 1.0g; $MgSO_4.7H_2O$- 0.5 g; $FeSO_4$-0.01g; soluble starch 20.0 g. The 100 ml of medium were taken in a 250 ml conical flask. The flasks were sterilized in autoclave at 121ºC for 15 mins and after cooling the flask will be inoculated with fungal cultures. The inoculated medium will be incubated at 30ºC in shaker incubator for different incubation time. At the end of the fermentation period, the culture medium was centrifuged at 5000 rpm for 15 mins (or 1700 rpm for 10 mins) and supernatant will be filtered. The supernatant was used for further estimation of enzyme activities. The enzyme activity was assayed using 3,5- dinitrosalicylic acid (Miller 1959; Tamang and Thapa 2006). Briefly, 0.1 ml of supernatant was incubated with 0.5 ml of soluble starch (1%, w/v) and 0.4 ml of buffer (0.1 M phosphate buffer for pH 7.0) and incubated at 40°C for 10 min. The reaction was terminated by the addition of 1 ml of 3, 5-dinitrosalicylic acid (DNS) reagent followed by boiling water bath for 5 min. After cooling, addition of 10 ml of distilled water was added and the liberated reducing sugars was estimated in UV-VIS Spectrophotometer at 660 nm absorbance of the resulting solution. One unit of amylase activity was defined as the amount of enzyme releasing 1 μmol of reducing sugars (glucose equivalents) per min under the assay condition at pH 7.0 at 40°C and was expressed as U/ml (Sunitha et al. 2012).

Screening of extracellular cellulase enzyme

Qualitative enzyme assay

Determination of cellulolytic activity was determined by Yeast Extract Peptone Agar medium containing 0.5% Carboxy-methylcellulose (CMC) was used for plate screening (Debnath et al. 2020). In addition, conidia from old culture were inoculated into center of plate. Plates were incubated at 30°C. After 3-5 days of fungal colony growth, the plates were flooded with 1.0% aqueous Congo red solution and destined with 1M NaCl for 15 mins. Appearance of yellow areas around the fungal colony in an otherwise red medium indicated cellulose activity (Shahriarinour et al. 2011).

Quantitative enzyme assay

The cultures were used to know their potential for cellulase production and activities. A volume of 100 ml of Czapek-Dox broth medium amended with 1% Carboxymethyl cellulose were distributed into separate 250 ml conical flasks. The pH of the medium was adjusted to 5. After autoclaving at 121°C and 15 lb. pressure, the fungal spores were inoculated into the conical flasks. The flasks were incubated at 30°C on a rotary shaker at 120 rpm for 4 days. The activity of Cellulase was measured by using the method of Miller 1959 (3, 5- dinitrosalysilic acid). The enzyme crude extract was prepared after 4 days, culture filtrate was collected, centrifuged at 6000 rpm for 15 mins and supernatant was used to the estimation extracellular cellulase enzyme. The reaction mixture was contained 0.5 ml of culture filtrate was mixed with 1.0 ml of substrate (1.0 % CMC in 0.2M acetate buffer (pH 5) in a test tube and incubated in a shaking water bath at 50°C for 1 h. The reaction was terminated by adding 3.0 ml of DNS reagent. The colour was then developed by boiling the mixture for 5 mins, followed by cooling

in cold water path. The optical density was taken at 640 nm against blank containing all the reagents minus the crude enzyme. One unit of the enzyme activity was defined as the amount of enzyme that released 1 µM of glucose under assay condition (Elad and Kapat 1999).

Screening of extracellular L-asparaginase enzyme

Qualitative enzyme assay

Screening of potential L-Asparaginase production were selected by plate assay method. MCD medium used for filamentous moulds were inoculated and incubated at temperature 30°C for 3-5 days. L-Asparaginase production was indicated by observing pink zone around the colonies and were selected for determination of enzyme activity. Control plates were maintained with $NaNO_3$ instead of L-asparagine as nitrogen source on MCD medium (Sanjotha 2017).

Quantitative enzyme assay

Assay of enzyme was performed by submerged fermentation and was carried out by using MCD broth media. The potential filamentous moulds were inoculated in 100 ml of suitable medium in culture flasks. The culture flasks were incubated at 30°C for 3 to 5 days. Uninoculated flasks were treated as controls. The fungal cultures were harvested by filtering through Whatman No. 2 filter paper. The estimation of enzyme activity was performed by crude enzyme obtained from culture filtrate. In this assay the rate of hydrolysis of L-Asparagine was determined by measuring the liberated ammonia by nesslerization. The cultures were centrifuged at 10000 rpm for 10 min, the reaction was carried by taking 0.5ml supernatant, 0.5ml 0.04M L-Asparagine and 0.5ml 0.05M tris HCl buffer (pH 7.2) make up the volume to 2.0 ml distilled water, incubated at 37ºC

for 30 min. After the incubation the reaction was stopped by addition of 0.5ml of 1.5M trichloroacetic acid (TCA). The reaction mixture was centrifugation at 8000 rpm, for 5 min at 40ºC to remove the precipitate. The 0.1 ml of supernatant, 3.75ml of distilled water and 0.2 ml of Nessler's reagent maintained at room temperature for 10 mins. Absorbance was measured at wavelength of 480 nm. Blank was prepared containing all the other reagents except enzyme. The ammonia liberated in the reaction was determined based on the standard curve obtained using ammonium sulfate. Enzyme unit: one international unit (IU) of L-asparaginase was defined as that amount of enzyme capable of producing 1micromole (μM) of ammonia per minute per ml [μmole/ml/min] at 37ºC, using asparagine as substrate.

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole of NH3 liberated})\ (2.5) \times 100\ (0.1)}{(30)\ (1)}$$

2.5 = Initial volume of enzyme mixture (ml)

0.1 = Volume of enzyme mixture used in final reaction (ml)

30= Incubation time (minutes)

1= Volume of enzyme used (ml)

Screening of extracellular lipase enzyme

Qualitative enzyme assay

The phenol red media was used in screening of lipase enzyme by phenol red. Isolated inoculates are grow in potato dextrose broth (PDB). Broth was collected aseptically in Eppendorf and centrifuged in 10,000 rpm for 20 min at 4˚C and collected a supernatant. Sterile Whatman filter disc (diameter 5.0 mm) as dipped in each supernatant and placed on top of chromogenic plates and incubated for 15-30 mins at 37-45˚C (Singh et al. 2006; Rajan et al. 2011; Lanka and Latha, 2015).

Quantitative enzyme assay

Filamentous moulds were grown in 250 ml flasks containing 100 ml of sterilized medium $(NH_4)_2SO_4$-3g, $MgSO_4$- 0.7g, NaCl- 0.5g, $Ca(NO_3)_2$-0.4g, KH_2PO_4 -1.0g, glucose-5.0g, yeast extract-1.0g at pH5) and were kept on shaking incubator at 30ºC and 110 rpm for 4 days. Olive oil was sterilized separately in dry oven and 1ml of it was added per 100 ml of sterile medium. The 10 ml of sample was taken after every 25 h for 4 days and sample was centrifuged at 5000 rpm for 20 min and supernatant was used for determining lipase activity. For this, 1.3 ml of olive oil, 1ml of phosphate buffer (0.066M at pH 7), 3 ml of supernatant and 1.5 ml of distilled water were shaken for 3 mins, then placed in an incubator shaker at 30ºC and at 150 rpm for 5 h. After incubation, 15 ml of ethanol was added to the reaction mixture, then 12.5 ml of diethyl ether was added to destroy the emulsion. The mixture obtained was titrated against 0.1N NaOH solution using Thymolphthalein as an indicator. One unit of lipase was defined as the amount of enzyme required to decrease the O.D. (optical density) value by 0.001 units per hour per ml of the liquid culture media containing the enzyme substrate under the assay conditions. One unit of lipase activity was defined as the amount of enzyme that liberated 1μmol fatty acid min-1 at 30°C and pH 7 under the assay conditions.

Screening of extracellular protease enzyme.

Qualitative enzyme assay

Gelatin agar media was used for the assessment of extracellular protease activity. After the required amount of the mycelial growth, plates were flooded with the reagent containing 15% of $HgCl_2$ and 20% of HCl. A visible zone was observed for the protease producing organisms (Fouda et al. 2015).

Quantitative enzyme assay

Protease activity was measured by degradation of casein. The 1ml of filtrate was added to 1ml of 1% (w/v) casein (pH-7.5) and incubated for 1 h at 45ºC. The reaction was stopped by adding protein precipitating agent, 3ml of 0.5M Trichloroacetic acid (TCA). Solutions were centrifuged at 5000 rpm for 30 min and absorption of filtrate was measured at 275 nm. One enzymatic unit represented the quantity of enzyme which liberates 1µg of tyrosine under enzyme assay condition.

Screening of extracellular Xylanase enzyme

Qualitative enzyme assay

Xylan agar was prepared with 1% Beechwood, 0.1% yeast extract and 1.6% agar and autoclaved. The test fungi were inoculated onto the agar plates**.** Clearance was considered as an indication of xylan utilization. Dilute iodine solution was used to stain the agar plates and a yellow opaque area around colonies indicated xylan degradation as compared to a reddish purple colour for undegraded xylan (Bailey et al. 1992; Choi et al 2005).

Quantitative enzyme assay

In quantitative screening, the filamentous moulds with zone of hydrolysis were selected and further screened in submerged state fermentation (SmF) for the production of xylanase. Erlenmeyer flasks (250 ml) containing 50 ml of liquid MSM medium with 0.1% of birch wood xylan as carbon source was inoculated with filamentous moulds and incubated at 30ºC and 120 rpm in an orbital shaker for 7 days. The flasks were withdrawn at each day and filtered through Whatman No.1 filter paper. The filtrate was centrifuged at 8000g at 4°C for 10 min and the supernatant was collected and used as

enzyme source for the assay of xylanase activity. Xylanase activity was determined by measuring the reducing sugar liberated from the xylan by 3, 5- dinitrosalicylic acid (DNS) (Miller 1959; Bailey et al. 1992). Crude enzyme extract was taken as xylanase source and assayed in 3.0 ml of reaction mixture containing 1% beechwood (prepared in 0.05 M Na-citrate buffer, pH 5.3). Reaction mixture is prepared by 1 ml of 0.05 M citrate buffer and with the addition of 1.0 ml of enzyme source and were incubated at 55°C for 10 min. The reaction was terminated by the adding 3.0ml of 3, 5-dinitrosalicylic acid (DNS) reagent and boiled for 15 mins in water bath (Miller 1959). 3 ml of distilled water was added to the mixture and the absorbance was read at 540 nm using a spectrophotometer after 10 min. One unit of xylanase activity (U) was defined as the amount of enzyme that liberates 1 µmol of reducing sugar - xylose per min under the standard assay conditions (Ramanjaneyulu et al. 2015).

Screening of antinutritive-degrading factor

Screening of Laccase

Qualitative enzyme assay

The filamentous moulds were screened for laccase enzyme by plate assay method using ABTS (2-2'-Azino-bis-[3-ethyl benzthiazoline-6-sulfonic acid]) and guaiacol as a substrates (Dhakar and Pandey 2013). The filamentous moulds was inoculated on different PDA agar plates containing 3 mM of ABTS and 4 mM of guaiacol) as individually and incubated at 30ºC for 7 days. The culture plates were observed for measure the color halo zone (Dhakar and Pandey 2013; Senthivelan et al. 2019).

Indicators and colours it produces in positive result

Sl. No.	Indicator	Positive reaction
1	ABTS	Green coloured zones around colony
2.	Guaicol	Dark brown coloured zone around colony

Quantitative enzyme assay

The filamentous moulds, grown on sterile discs in PDF plates after 7 days, were cut into small discs (5 mm size) (Senthivelan et al. 2019). About 4 PDA agar discs containing fungi mycelia were transferred to 250 ml flasks containing 100 ml of laccase production with the media compositions of (g/l): glucose-20; peptone-5; ammonium tartarate-10; yeast extract-1.0; KCl-0.5; KH_2PO_4-1; $MgSO_4.7H_2O$-0.5; $CuSO_4.5H_2O$ 0.25; and pH adjusted to 5.5 (Senthivelan et al. 2019). All the culture flasks were incubated at 30°C for 7 days on a rotary shaker at 120 rpm. After 7 days of incubation samples of each flask withdrawn from culture flasks and centrifuged at 10000 rpm for 15 mins. Solids were discarded and the supernatant was assayed for enzyme activity. The measurement of laccase activity was measured by spectrophotometer using guaiacol as a substrate. The reaction mixture consists of 3 ml of 100 mM of guaiacol dissolved in 10% acetone in sodium acetate buffer and 1 ml of culture filtrate (crude laccase). Absorbance was recorded at 470 nm, following 10 min of incubation at room temperature. One unit of laccase activity was defined as the amount of enzyme catalyzing the substrate (guaiacol) for the production of 1 ml of colored product per min per ml (Senthivelan et al. 2019).

Screening of Phytase

Qualitative enzyme assay

Isolates were inoculated on plates of phytase screening medium (PSM) and incubated at 30°C for 3-4 days (Bae et al. 1999). The PSM plates were flooded with 2% aqueous cobalt chloride solution. After 5 mins of incubation at room temperature, the cobalt chloride solution was replaced with freshly prepared solution containing equal volumes

of ammonium molybdate solution (6.25%) and ammonium vanadate solution (0.42%). Following 5 minutes incubation, solution of ammonium molybdate and ammonium vanadate was removed and plates examined for clearing zone of hydrolysis (Lee et al. 2005; Ahmad et al. 2018).

Quantitative enzyme assay

Each of the filamentous moulds strains was grown in 50 ml of phytase specific medium and incubated at 30°C at 200 rpm in a rotary shaker incubator for 5-6 days (Quan et al. 2001). Supernatant from 1 ml of culture after centrifugation at 6000 g for 10 min at 4°C, the clear supernatant was used as the source of extracellular phytases and used for the phytase activity assay. The Phytase activity was determined by measuring the amount of liberated inorganic phosphate. The reaction mixture consisted of 0.9 ml of acetate buffer contains 0.5% sodium phytate prepared in sodium acetate buffer (0.2 M, pH 5.5) and 0.1 ml of supernatant. After incubation 30 min s at 45°C, the reaction was quenched with 1ml of 10% trichloroacetic acid. A 1.0 ml of the colorimetric reagent, prepared from 10% (w/v) ammonium molybdate solution in 5 M sulfuric acid solution, were added to the test tubes. The reagent was prepared at the time of use by mixing 10% (v/v) of the solution to 5% (w/v) ferrous sulfate and deionized water. The absorbance was measured at 700 nm. The absorbance values were correlated with a standard curve of KH_2PO_4. The enzymatic activity analyses were performed in triplicate. An enzyme unit (U) was defined as the amount of enzyme required to release 1 μmol of inorganic phosphate per minute under the assay conditions. The liberated phosphate ions were quantified by 500μl of 10N H_2SO_4, 10% ammonium molybdate and 5% $FeSO_4$. After 30 mins of incubation at 45°C, absorbance was measured at 660

nm. One enzyme unit (IU) was defined as the amount of enzyme liberating 1 μmol of inorganic phosphate in 1 min under the assay conditions (Quan et al. 2001).

Screening of tannase

Qualitative enzyme assay

Screening of tannase was conducted following the method described by Bradoo et al. (1996). The solution of tannic acid was sterilized separately by passing through a membrane filter (pore size 0.22 μm) and was added to the Czapek Dox's minimal medium at a final concentration of 1%. Point inoculations were carried out and plates were incubated at 30°C for 3-4 days. The diameter of the hydrolytic zone was measured at three points and the average were calculated. The filamentous moulds showing a zone of tannic acid hydrolysis were considered as tannase producers. The potent tannase producers were further tested quantitatively for the amount of enzyme produced in broth.

Quantitative enzyme assay

Quantitative enzyme assay of the tannase-producing filamentous moulds were cultured under submerged fermentation using 250 ml flasks containing 50 ml of sterilized modified Czapek dox minimal medium (pH 5.0) (Cavalcanti et al. 2017). Filtered-sterilized 1% tannic acid was added to the autoclaved medium. Each strain was inoculated into the culture medium and maintained at 30°C in shaker an incubator for 3-4 days at 120 rpm. After the incubation period, the culture filtrate (through Whatman No.1 paper) was analyzed for tannase activity. The reaction mixture (1 ml) contained 250 μL 1% tannic acid (in phosphate buffer, pH 6.0), 500 μL of phosphate buffer (pH 6.0) and 250 μL of the culture filtrate and the mixture was incubated at 40°C for 30 min

in a water bath. The reaction was stopped by adding 1 ml 2% bovine serum albumin (BSA) solution. In the control, BSA was added in the mixture prior to incubation. All tubes were left for 20 mins at room temperature to precipitate residual tannins and were centrifuged at 3000 g for 20 mins. The tannase activity in the supernatant was estimated after appropriate dilution and reading absorbance at 260 nm (the optimal absorption of gallic acid) against the control in a UV spectrophotometer. One enzyme unit was the amount of enzyme that liberates 1 μmol gallic acid per ml per min under standard assay conditions (Cavalcanti et al. 2017).

RESULTS

SURVEY ON DRY STARTER CULTURES OF NORTH EAST INDIA

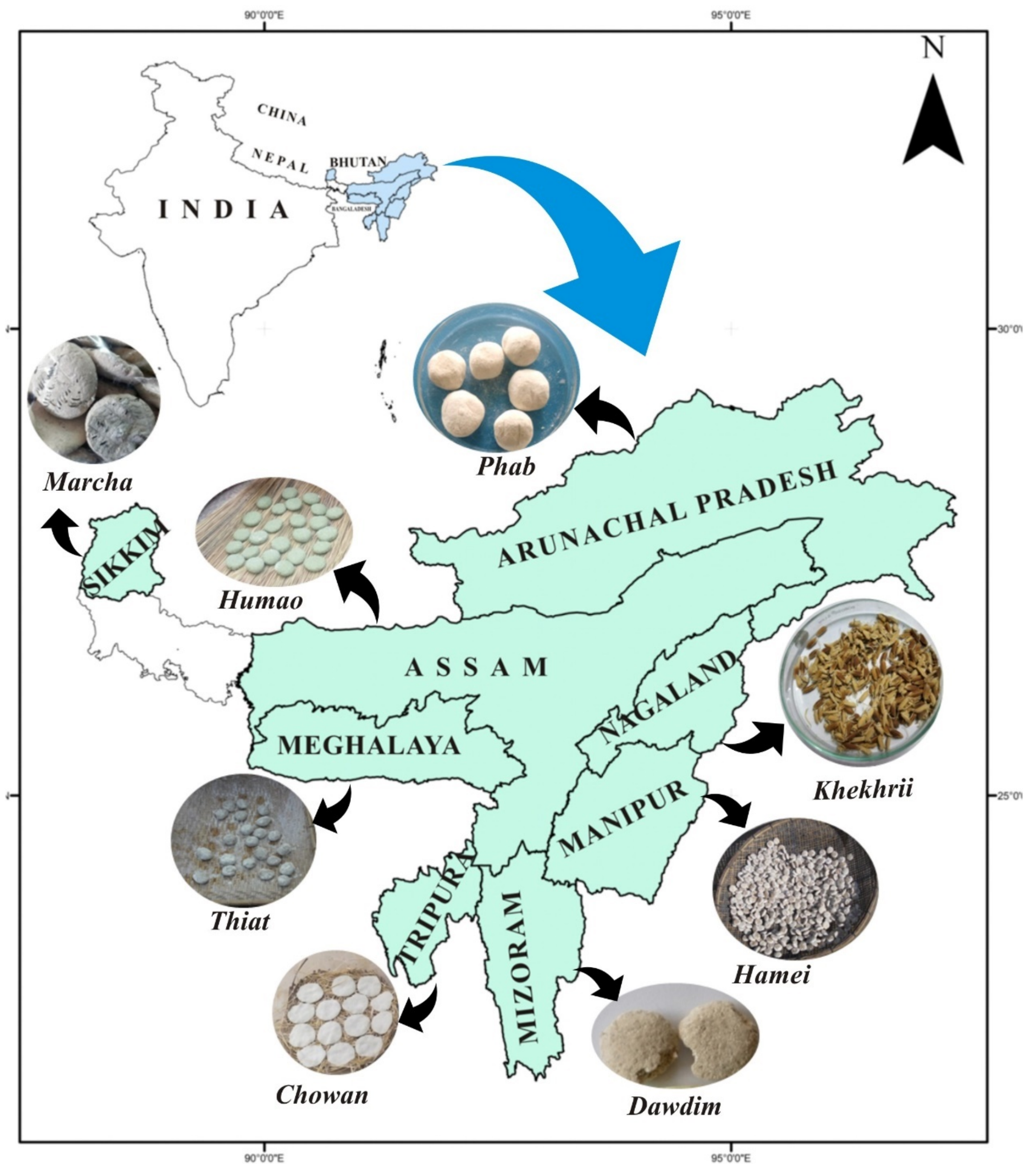

Figure: 1. Map showing different collection sites of traditionally prepared starters of North East India.

Eight states of India viz. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, located in North East regions (Fig. 1), were surveyed extensively and sought information on traditional methods of preparation, use of starters for production of alcoholic beverages and socio-economy of ethnic fermented amylolytic starters using Questionnaire (Table A). Based on compilation of questionnaire we documented eight different types of traditionally prepared starters of North East India viz. *Marcha* of Sikkim, *Thiat* of Meghalaya, *Humao* of Assam, *Hamei* of Manipur, *Chowan* of Tripura, *Phut* of Arunachal Pradesh, *Dawdim* of Mizoram and *Khekhrii* of Nagaland (Fig. 2). Methods of preparation and uses of each starter are were documented as below.

Figure: 2. Traditionally prepared starters of North East India: *Marcha* of Sikkim, *Thiat* of Meghalaya, *Humao* of Assam, *Hamei* of Manipur, *Chowan* of Tripura, *Phut* of Arunachal Pradesh, *Dawdim* of Mizoram and *Khekhrii* of Nagaland.

MARCHA

Marcha is dry, creamy white, flat and solid ball like cake used to ferment cereals to produce different types of ethnic mild-alcoholic beverages in Sikkim.

Ingredients: glutinous rice (*Oryza sativa*), wild herbs such as roots of 'guliyo jara' or 'chitu' (*Plumbago zeylanica*) leaves of 'bheemsen paate' (*Buddleja asiatica*), flower of 'sengreknna' (*Vernonia cinerea*), ginger and red dry chili (2-3 pieces).

Indigenous knowledge of preparation

During *marcha* preparation glutinous rice (*Oryza sativa*) is soaked in water for 8-12 h (overnight) at room temperature. After soaking glutinous rice is crushed in a foot driven heavy weight wooden mortar pestle. Various wild herbs such as roots of 'guliyo jara' or 'chitu' (*Plumbago zeylanica*) leaves of 'bheemsen paate' (*Buddleja asiatica*), flower of sengreknna' (*Vernonia cinerea*), ginger and red dry chili (2-3 pieces) are crushed and added to the powdered glutinous rice. Powdered mixture of glutinous rice and wild parts of herbs are then made into paste by adding water and kneaded into flat cakes of different sizes and shapes are made. These newly prepared *marcha* cakes are then dusted with the old powdered *marcha* which are used as source of an inoculum. The freshly prepared *marcha* cakes are then placed individually on the leaves of ferns, locally called '*pirey fern'* (*Glaphylopteriolopsis erubeseens*) and cover with dry ferns and jute bags. Then cakes are left for 2-3 days, the longer period being used in winter season. After incubation the ferns and jute bags are removed and the *marcha* cakes are collected and sun dried for 2-3 days (Fig. 3). Dried *marcha* cakes are used to prepare alcoholic beverages such as *Kodo ko jaanr* (fermented finger millet beverage), *Bhaati jaanr* (fermented rice beverages) and *Raksi* (distilled liquor). Some people sell *marcha* in the local markets for their livelihood.

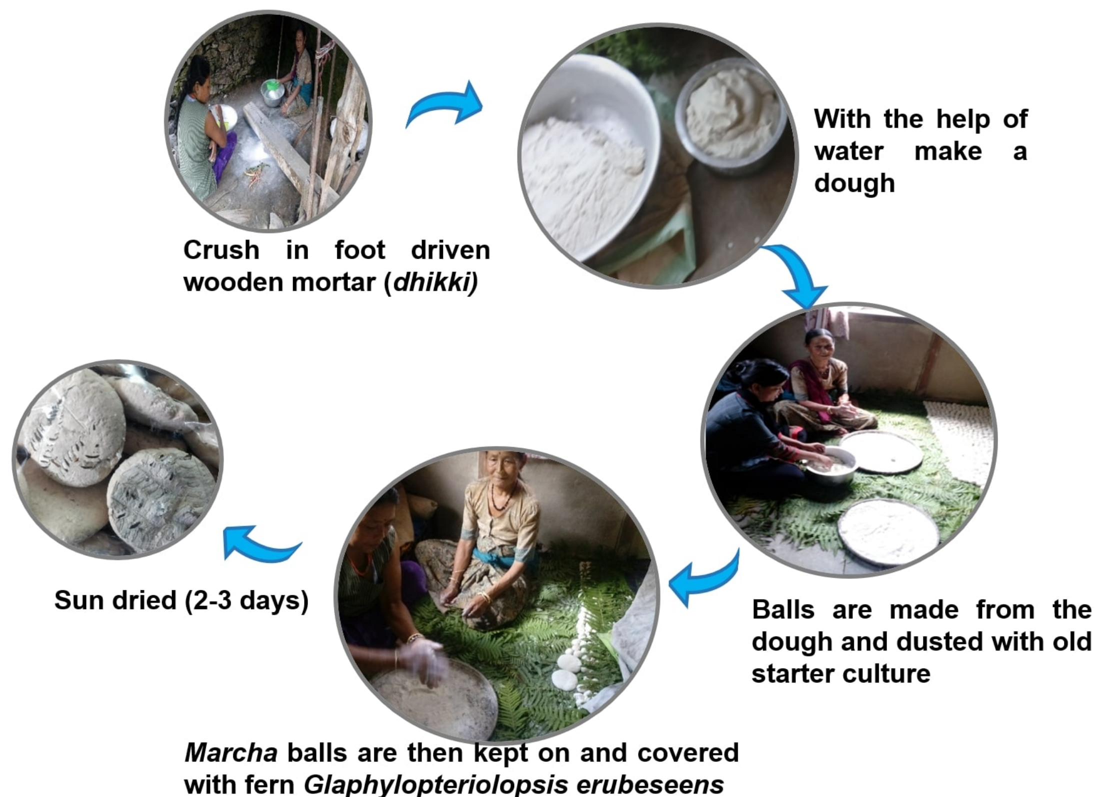

Figure: 3. Flowsheet diagram for preparation of *marcha* in Sikkim.

THIAT

Thiat is a traditionally prepared dry starter of Meghalaya by the *Pnar* ethical community of Jaintia hills which is used to produce mild alcoholic beverage locally known as *kiad*.

Ingredients: glutinous rice, powdered *khaw-iang-/hawiang* plants leaves, *sla-pashor* (Leaves of banana).

Indigenous knowledge of preparation

Glutinous rice is washed, soaked and then powdered in *thlong*-a mortar made of hard wood of *schima wallichii* during *thia*t preparation (Fig. 4). The rice powder is mixed with powdered leaves of *khaw-iang-/hawiang* (*Amomum aromaticum* Roxb) plants with clean water and small flat to round balls are made. Then balls are kept for fermentation 2-3 days in *malieng* (a round basket) and covered with *sla-pashor* (leaves of banana). After fermentation, freshly prepared *thiat* is sun dried for 3-5 days and is stored. Some ethnic people sell *thiat* in local markets of Meghalaya.

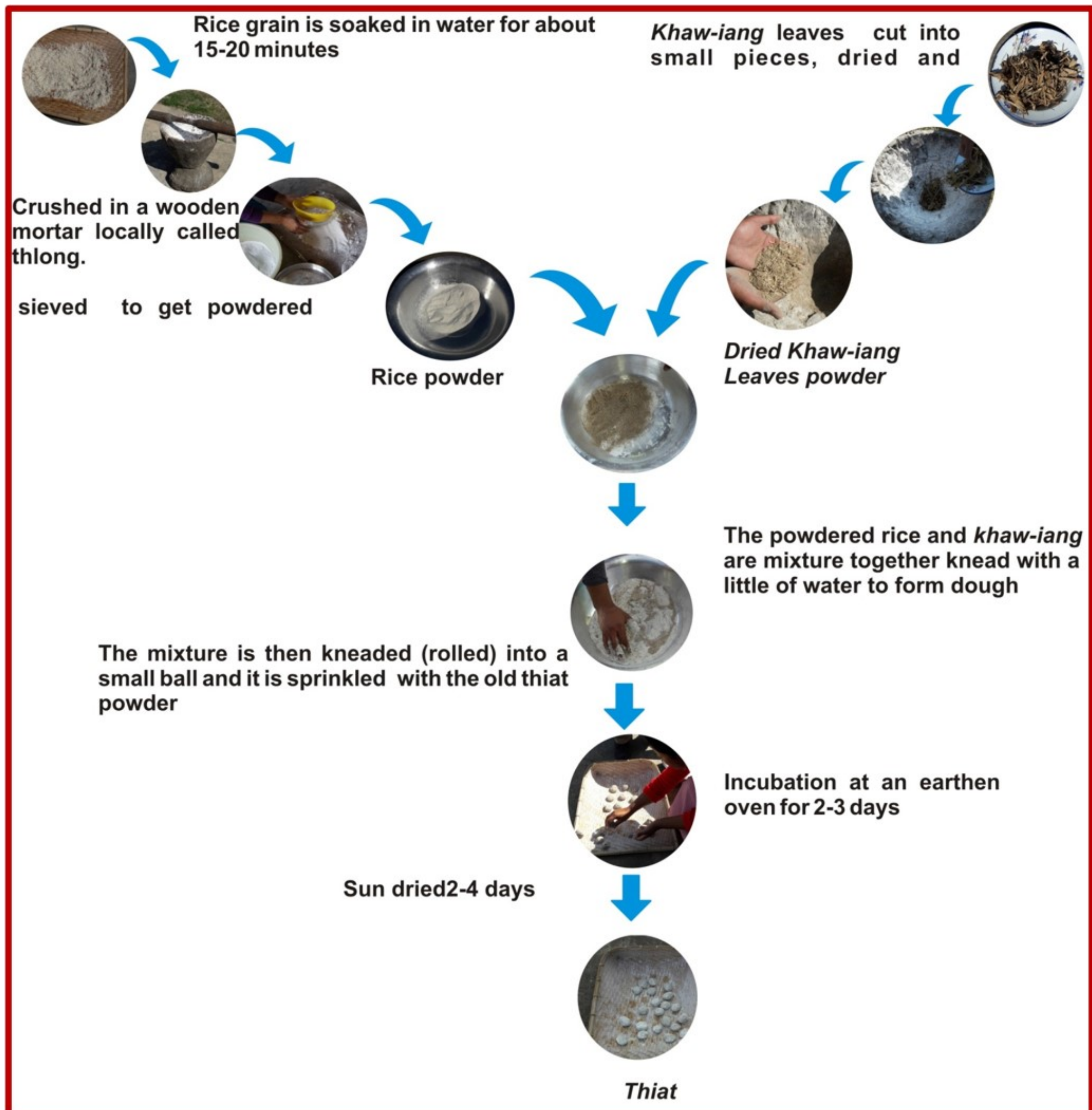

Figure: 4. Flowsheet diagram for preparation of *thiat* in Meghalaya.

HUMAO

Huamo/*emao* is a traditionally prepared rice-based ethnic starter culture of Assam. It is prepared by *Bodo* and *Ahom* communities of Assam. *Humao* is commonly used for the preparation of *jou/judima/zu,* mild alcoholic beverage in rural parts of Assam.

Ingredients: glutinous rice (*Oryza sativa)* leaves of *banana, lwkwna, dong-phang-rakhep* (*Scoparia dulcis*) and dry barks of *Albizia myriophylla*

Indigenous knowledge of preparation

Local glutinous rice (*Oryza sativa*) is soaked in water for 2-3h and mixed with various types of plants like leaves of banana, *Dong-Phang-Rakhep* (*Scoparia dulcis*), *Khantal* and *Lwkwna* leaves. Ingredients are then ground into the wooden mortar pestle (this group of traditional apparatus is locally called *gaihe*n and *ual*). Then the powdered rice is sieved in *sandri* (traditional bamboo sieve), little amount of water is added to make a thick paste or dough. Different sizes of small round to oval cakes are prepared from dough, which is then dusted with previously prepared *humao*, used as the source of an inoculum. Once the fresh *humao* cakes are prepared they are covered by paddy straw inside the bamboo basket and fermented for 2-3 days. Once the natural fermentation is completed, the *humao* cakes are sun-dried naturally for 2-3 days (Fig. 5). Sometimes, this natural drying process continues for a couple of weeks before *humao* cakes get tough and ready to be used for alcoholic beverage preparation as well as market sales.

Figure: 5. Flowsheet diagram for preparation of *humao* in Assam.

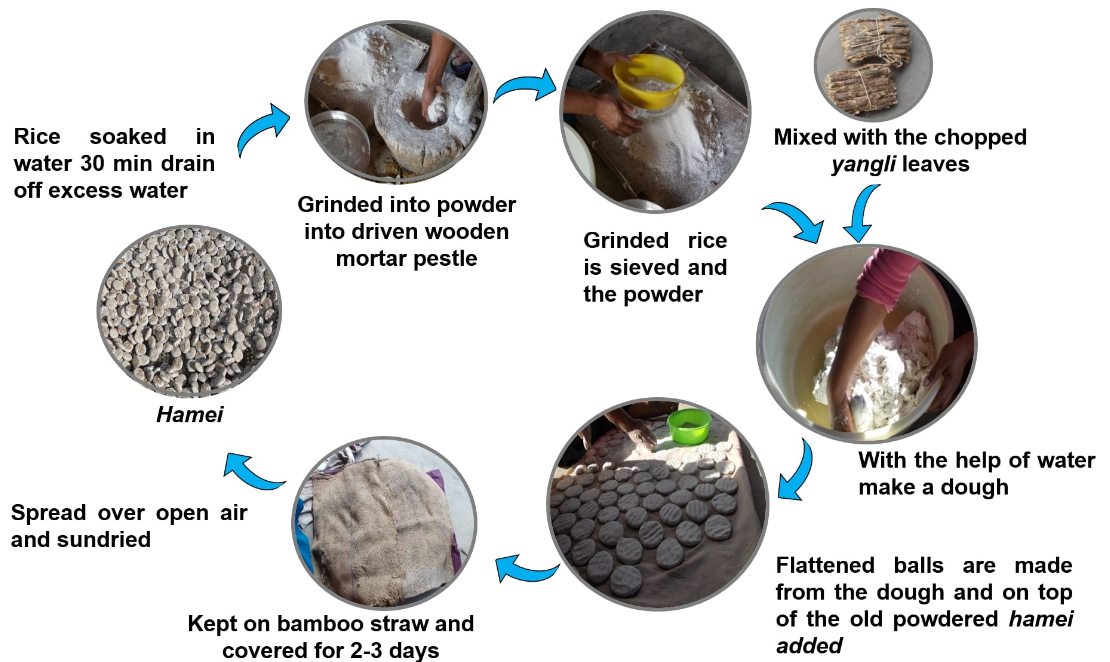

Figure: 6. Flowsheet diagram for preparation of *hamei* in Manipur.

HAMEI

Hamei is a dry and round to flattened traditionally prepared starter of Manipur. It is used in Manipur for the preparation of rice-based alcoholic beverage based called *atingba* and its distillate part is known as *yu.*

Ingredients: glutinous rice (*Oryza sativa),* bark of *yangli (Albizia myriophylla* Benth.*)*

Indigenous knowledge of preparation

Hamei is made from local glutinous rice varieties, which are soaked in water for 30 mins to 1 h and dried for 15 mins to remove excess water. The rice is pounded in a wooden mortar and powdered rice is combined with '*yangli*' (*Albizia myriophylla* Benth.) powdered bark and added 1% of previously prepared *hamei.* Water is added to make thick dough and the round to flat balls are made from the dough and kept in paddy husk in a bamboo basket, covered by sack clothes and fermented for 2-3 days at room temperature. After fermentation, cakes are sun-dried for 2-3 days (Fig. 6). It was observed during survey that *hamei* is prepared during the summer (May-July), and can be kept at room temperature for more than a year.

CHOWAN

Chowan is a traditionally prepared starter of Tripura and is used to prepare alcoholic beverages in Tripura.

Ingredients: glutinous rice (*Oryza sativa),* wild herbs

Indigenous knowledge of preparation

Soaked glutinous rice is mixed with leaves and roots of various kinds of herbs during the preparation of *chowan.* This mixture is dusted with 1-2% of previously prepared powdered *chowan*. The above mixture is then made into dough by mixing with water and making the dough into varying shapes and size from round to flat and oval cakes.

Cakes are kept in paddy straw inside the bamboo basket and fermented for 2-3 days, and then sundried for 3-7 days (Fig. 7).

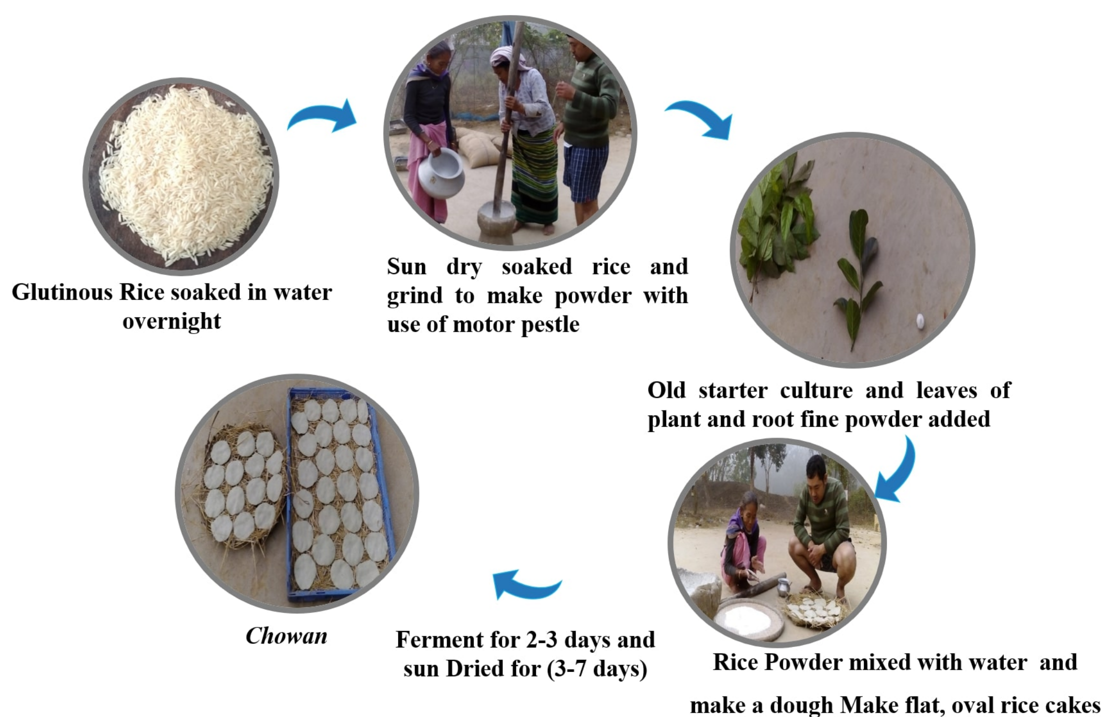

Figure: 7. Flowsheet diagram for preparation of *chowan* in Tripura.

PHUT

Phut is a traditional starter culture of Arunachal Pradesh prepared by the Tagin community, which is round to flat white to dusty colour. Apatani and Nyshing communities also prepare *phut*.

Ingredients: glutinous rice (*Oryza sativa),* "*Nakail*" (*Cinnamomum glanduliferum* Mesissn.) and *Ctuepatti* (*Cissampelos pareira* Linn. and *Khanoba* (*Clerodenderon viscosum* Vent.).

Indigenous knowledge of preparation

The glutinous rice is soaked and powdered by using wooden mortar and pestle and grinded into fine powder. Few leaves of the "*Nakail*" (*Cinnamomum glanduliferum* Mesissn.) and *Ctuepatti* (*Cissampelos pareira* Linn. and *Khanoba* (*Clerodenderon*

viscosum Vent.) along with 5-7 old powdered starter cakes of *phut* are added to rice flour and mixed properly. The mixture is made into dough by adding water, and is shaped as desirably, wrapped in paddy straw inside basket and fermented for 1-2 days. Then these are sun-dried for 5-10 days and store up to 6 months for further use and sale (Fig. 8). *Phut* is used to fermented rice to make traditional alcoholic beverage of Arunachal Pradesh called *Apong* and *Madua Apong*. *Phut* is mostly prepared by women during rituals and other ceremonies.

Figure: 8. Flowsheet diagram for preparation of *phut* in Arunachal Pradesh.

DAWDIM

Dawdim is a traditionally prepared starter culture of Mizoram and is used in preparation of local alcoholic beverages.

Ingredients: glutinous rice (*Oryza sativa),* local herbs

Indigenous knowledge of preparation

During preparation, soaked local rice varieties are crushed to make fine rice powder, combined with local herbal leaves and 2% old powdered *dawdim.* The mixture is made into thick dough by adding water and kneaded into flat and oval cakes of various sizes and shapes, wrapped in fern leaves in bamboo baskets, then covered with jute bags and placed over the earthen kitchen oven for 1-3 days for fermentation. These freshly prepared cakes are sun dried for 3-5 days (Fig. 9).

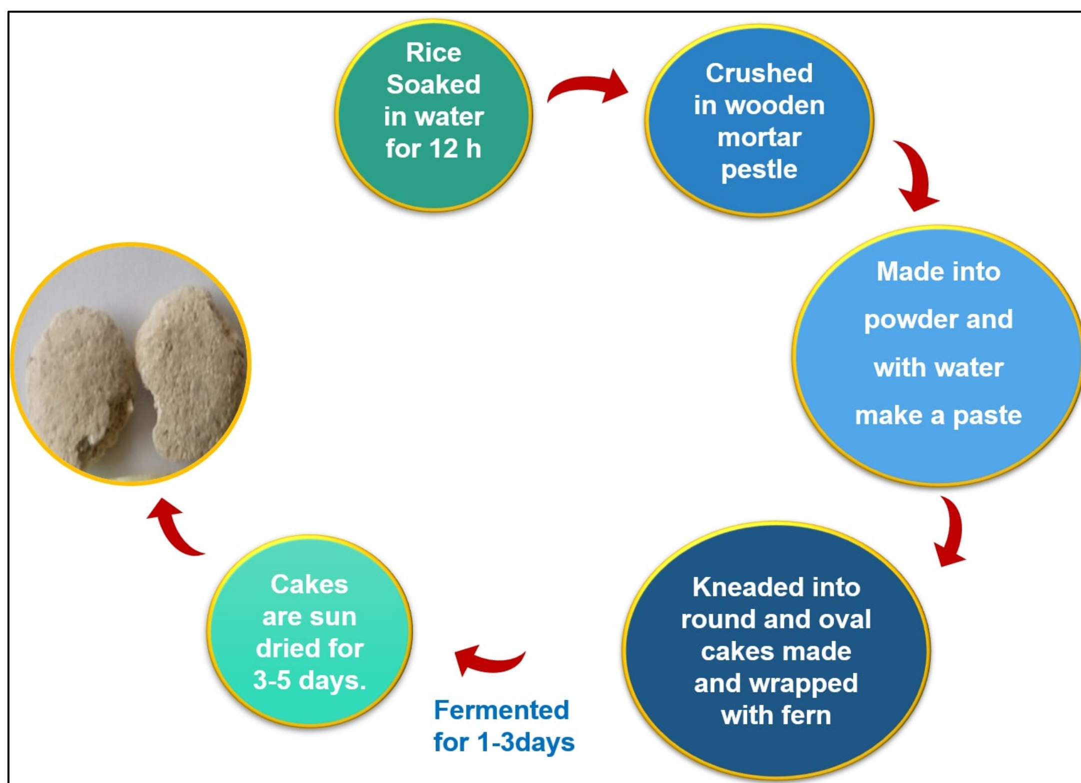

Figure: 9. Flowsheet diagram for preparation of *dawdim* in Mizoram.

KHEKHRII/KHRIE

It is a traditionally prepared starter in Nagaland which is used to prepare mild, local alcoholic beverage locally called *zutho.* This is the only starter in North-East India that which is prepared by using fermenting un-hulled glutinous rice grains

Ingredients: glutinous rice (*Oryza sativa*)*, Khreihenyii* leaves

Indigenous knowledge of preparation

Unhulled glutinous rice (*dhan*) is washed twice or thrice with water, and then soaked for 2-5 days in water. It is then kept and covered with *Khreihenyii* leaves (local leaves) and allowed to germinate 2-3 days in summer and 4-5 days in winter season. Once the germination is up to about half an inch in length, the germinated rice is sprouted and the sprouted rice is naturally sun dried and powdered. The powdered sprouted rice is again sun dried and then it is ready to use as *khekhrii* for the preparation of alcoholic beverage and for the sell too (Fig. 10).

Figure: 10. Flowsheet diagram for preparation of *khekhrii* in Nagaland.

FUNGAL LOAD, MOISTURE and pH

We collected 40 samples of traditionally prepared starters viz. *marcha* (8 samples), *thiat* (4), *humao* (7 samples), *hamei* (3 samples), *chowan* (4 samples), *phut* (6 samples), *dawdim* (8 samples) and *khekhrii* (5 samples) from different places of North East India (Table 1). The fungal populations in traditionally prepared starters of North East India were recorded 2.5 to 7.9 x 10^5 cfu/g (Table 1). Fungal cfu/g (10^5 cfu/g) count of *marcha* was in the range between 4.8-5.1, *thiat* 4.5-5.1*, humao* 4.3-5.3, *hamei* 2.5-3.2, *chowan* 3.0-3.4, *phut* 4.9-5.9, *dawdim* 7.1-7.9, and *khekhrii* 5.7-6.8, respectively. The moisture contents of *marcha* was in the range between 10.1-12.1%, *thiat* 8.7-10.0%*, humao* 8.8-10.6%, *hamei* 8.0-9.6%, *chowan* 9.0-9.3%, *phut* 11.4-11.8%, *dawdim* 13.1-13.9% and *khekhrii* 12.3-13.1%, respectively. The pH of *marcha* was in the range between 4.9-5.7 and that of *thiat* 4.5-5.0*, humao* 4.6-5.2, *hamei* 4.1-5.4, *chowan* 5.4-5.9, *phut* 5.5-5.7, *dawdim* 6.1-6.3, and *khekhrii* 5.5-5.9, respectively.

Table 1: Sample collection details, fungal load, moisture content and pH of dry starters of North East India						
Sample (number of samples)	**Region**	**Collection Site**	**Altitude (Metre)**	**Fungal load (cfu/g x 10^5)**	**Moisture content (%)**	**pH**
Marcha (8)	Sikkim	Gangtok	1637	5.0 (4.8-5.1)	11.6 (10.1-12.1)	5.2 (4.9-5.7)
		Basilakha	906			
		Pakyong	1341			
		Recabe	1072			
Thiat (4)	Meghalaya	Shillong	1550	4.8 (4.5-5.1)	9.4 (8.7-10.0)	4.7 (4.5-5.0)
		Nongrem	1547			
Humao (7)	Assam	Kokrajhar	49	4.6 (4.3-5.3)	9.7 (8.8-10.6)	4.9 (4.6-5.2)
		Jorhat	95			
		Sivsagar	93			
		Moran	100			
Hamei (3)	Manipur	Kangchup	773	2.6 (2.5-3.2)	8.5 (8.0-9.6)	4.6 (4.1-5.4)
		Kakching	769			
		Phayeng	813			
Chowan (4)	Tripura	Bangsul	116	3.1 (3.0-3.4)	9.1 (9.0-9.3)	5.6 (5.4-5.9)
		Dharmanagar	98			
Phut (6)	Arunachal Pradesh	Doimukh	152	5.6 (4.9-5.9)	11.2 (11.4-11.8)	5.4 (5.5-5.7)
		Pasighat	155			
		Itanagar	361			
		Banderdewa	462			
		Nirjuli	151			
Dawdim (3)	Mizoram	Saitual	438	7.4 (7.1-7.9)	13.7 (13.1- 13.9)	6.2 (6.1-6.3)
Khekhrii (5)	Nagaland	Kohima	1092	6.0 (5.7-6.8)	12.8 (12.3-13.1)	5.6 (5.5-5.9)

MORPHOLOGICAL CHARACTERIZATION

We isolated 131 total filament moulds isolates from 40 different samples of traditionally prepared dry starters (*marcha*, *thiat*, *humao*, *hamei*, *chowan*, *phut*, *dawdim* and *khekhrii*) collected from eight states of North East India. All 131 filamentous moulds isolates were studied macroscopically by observing the colony characters (surface colour, reverse side colour, shape, size and hyphae) and microscopically by observing fruiting bodies, vegetative and asexual stages using stereomicroscope (Table 2-10). Based on the morphological characteristics (such as colour, texture, size and appearance of colony), microscopic characteristics (hyphae, sporangia, sporangiospores, chlamydospores, conidia, conidiophore and rhizoid structure), 44 representative fungal isolates were grouped (7 isolates from *marcha*, 5 from *thiat*, 6 from *humao*, 2 from *hamei*, 5 from *chowan*, 6 from *phut,* 6, from *dawdim* and 7 from *khekhrii*) (Table 10). *Mucor*, *Rhizopus*, *Aspergillus, Penicillium,* and *Cladosporium* and few unidentified basidiomycetes fungi were tentatively identified on the basis of detailed morphological characters using the taxonomical keys described by Samson et al. (2004) and Pit and Hocking (2009).

Table 2: Macroscopic and microscopic characteristics of filamentous moulds isolated from *marcha* samples of Sikkim.								
Sample	**Isolate code**	**Macroscopic characteristics**			**Microscopic Characteristics**			**Tentative identification**
		Colour	**Pigment**	**Exudate**	**Mycelia**	**Conidia**	**Spore structure**	
Marcha	SMM-1; SMM-12; SMM-34	Colonies diameter of 2-5 cm within 5 days with olive green color.	No	Yes	Septate	Phialosporae	Conidiophores upright, simple, aseptate and clavate with swelling. Phialides borne directly on the vesicles and radiating from the entire surface; conidia 1-celled, globose, often variously coloured in mass.	*Aspergillus flavus*
	SMM-3; SMM-6; SMM-13; SMM-14; SMM-20; SMM-25; SMM-28; SMM-29; SMM-32	Colonies raise fast, floccose, light yellow to greyish up to 15mm height	No	No	Aseptate	Sporangiospore s	Sporangiophores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.	*Mucor*
	SMM-10; SMM-18; SMM-21	6-7 days colonies show maximum diameter 8.0 mm initially woolly, white, later turn to yellowish, reverse white, or yellow	No	No	Septate	Arthroconidia	Hyphae dichotomously branched, 3-4.5µm diameter, and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.	Not Identified
	SMM-2; SMM-4; SMM-17; SMM-19;	Color of colony is white, slowing turning to grey to blackish after 2-3 days	No	No	Aseptate	Sporangio-spores	Stolon well developed subhyaline to light brown or grayish brown, aseptate, Rhizoids are branched and variable in length. Sporangiophore arising from stolon and directly from aerial hyphae, solitary or 2-3 in	*Rhizopus*

Table 2: Macroscopic and microscopic characteristics of filamentous moulds isolated from *marcha* samples of Sikkim.								
Sample	Isolate code	Macroscopic characteristics			Microscopic Characteristics			Tentative identification
		Colour	Pigment	Exudate	Mycelia	Conidia	Spore structure	
	SMM-23; SMM-27;						groups, straight to slightly curved. Sporangiospores (20-100µm diameter).	
Marcha	SMM-5; SMM-8; SMM-15; SMM-16; SMM-22; SMM-24; SMM-26; SMM-30; SMM-31; SMM-33; SMM-35	Colonies diameter of 3-6 cm within 8 days. Colonies are velvety to floccose with yellow green shade	Yes	Yes	Septate	conidiophores	Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled conidia.	*Penicillium*

Table 3: Macroscopic and microscopic characteristics of filamentous moulds isolated from *thiat* samples of Meghalaya.								
Sample	**Isolate code**	**Macroscopic characteristics**			**Microscopic Characteristics**			**Tentative identification**
		Colour	**Pigment**	**Exudate**	**Mycelia**	**Conidia**	**Spore structure**	
Thiat	MTM-1; MTM-3; MTM-11;	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	No	Aseptate	sporangiospore s	Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor*
	MTM-2; MTM-4; MTM-5; MTM-7;	Color of colony is white, slowing turning to grey to blackish after 2-3 days	No	No	Aseptate	Sporangiospore s	Stolon well developed subhyaline to light brown or grayish brown, aseptate, Rhizoids are branched and variable in length. Sporangiophore arising from stolon and directly from aerial hyphae, solitary or 2-3 in groups, straight to slightly curved. Sporangiospores (20-100µm diameter).	*Rhizopus*
	MTM-6; MTM-8	Colonies diameter of 3-6 cm within 8 days. Colonies are velvety to floccose with yellow green shade	Yes	Yes	Septate	Conidiophores	Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled conidia.	*Penicillium*
	MTM-12; MTM-16	6-7 days colonies show maximum diameter 8.0 mm initially woolly, i white, later turn to yellowish, reverse white, or yellow	No	No	Septate	Arthroconidia	Hyphae dichotomously branched, 3-4.5µm diameter, and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.	Not Identified

Table 4: Macroscopic and microscopic characteristics of filamentous moulds isolated from *humao* samples of Assam.

Sample	Isolate code	Macroscopic characteristics			Microscopic Characteristics			Tentative identification
		Colour	Pigment	Exudate	Mycelia	Conidia	Spore structure	
Humao	AEM-8; AEM-13; AXM-1; AXM-2	Colonies green to blue colonies with yellowish shades, velutinous texture	Yes	Yes	Septate	Conidiophores	Hyaline conidiophores smooth-walled stipes, vesicles (6.0µm-17µm wide), metulae (2µm-3µm) and phialides (2µm–4.5µm) in size. Spherical conidia 2.0µm to 3.5µm in diameter, hülle cells present	*Aspergillus*
	AEM-2; AEM-4; AEM-6; AEM-10; AMM-3; AMM-5; AMM-6; AMM-7; AMM-9;	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	No	Aseptate	Sporangio-spores	Sporangiosphores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor*
	AEM-1; AEM-9; AEM-12	Colonies diameter of 3-6 cm within 8 days. Colonies are velvety to floccose with yellow green shade	Yes	Yes	Septate	Conidiophores	Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled conidia.	*Penicillium*
	AEM-3; AEM-5; AEM-7;	Color of colony is white, slowing turning to grey to blackish after 2-3 days	No	No	Aseptate	Sporangio-spores	Stolon well developed subhyaline to light brown or grayish brown, aseptate, Rhizoids are branched and variable in length. Sporangiophore arising from stolon and directly from aerial hyphae, solitary or 2-3 in groups, straight to slightly curved. Sporangiospores (20-100µm diameter).	*Rhizopus*

Table 5: Macroscopic and microscopic characteristics of filamentous moulds isolated from *hamei* samples of Manipur.								
Sample	**Isolate code**	**Macroscopic characteristics**			**Microscopic Characteristics**			**Tentative identification**
		Colour	**Pigment**	**Exudate**	**Mycelia**	**Conidia**	**Spore structure**	
Hamei	MHM-1; MHM-6; MHM-3; MHM-8; MHM-14	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	No	Aseptate	Sporangio-spores	Sporangiosphores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor*
	MHM-10; MHM-12; MHM-15	1-2 cm diameter within 6-7 days, conidiophore with blue green in colour. Reverse colony yellow to orange	Yes	Yes	Septate	Conidiophores	Conidiophores 60-100µm, smooth walled. Metulae bearing 4-5 phialides. Conidia are globose, smooth walled, hyaline.	*Penicillium*

Table 6: Macroscopic and microscopic characteristics of filamentous moulds isolated from *chowan* samples of Tripura.

Sample	Isolate code	Macroscopic characteristics				Microscopic Characteristics		Tentative identification
		Colour	Pigment	Exudate	Mycelia	Conidia	Spore structure	
Chowan	TCM-9	Colonies green to blue colonies with yellowish shades, velutinous texture	Yes	Yes	Septate	Conidiophores	Hyaline conidiophores smooth-walled stipes, vesicles (6.0µm-17µm wide), metulae (2µm-3µm) and phialides (2µm–4.5µm) in size. Spherical conidia 2.0µm to 3.5µm in diameter, hülle cells present	*Aspergillus*
	TCM-4	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	NO	Aseptate	Sporangio-spores	Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor*
	TCM-1; TCM-5; TCM-6; TCM-8	6-7 days colonies show maximum diameter 8.0 mm initially woolly, i white, later turn to yellowish, reverse white, or yellow	No	No	Septate	Arthroconidia	Hyphae dichotomously branched, 3-4.5µm diameter, and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.	Not Identified
	TCM-12	Colonies diameter of 3-6 cm within 8 days. Colonies are velvety to floccose with yellow green shade	Yes	Yes	Septate	conidiophores	conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled conidia.	*Penicillium chrysogenum*
	TCM-2; TCM-3; TCM-7; TCM-11	Color of colony is white, slowing turning to grey to blackish after 2-3 days	No	No	Aseptate	Sporangiospore s	Stolon well developed subhyaline to light brown or grayish brown, aseptate, Rhizoids are branched and variable in length. Sporangiophore arising from stolon and directly from aerial hyphae, solitary or 2-3 in groups, straight to slightly curved. Sporangiospores (20-100µm diameter).	*Rhizopus oryzae*

Table 7: Macroscopic and microscopic characteristics of filamentous moulds isolated from *phut* samples of Arunachal Pradesh.

Sample	Isolate code	Macroscopic characteristics			Microscopic Characteristics			Tentative identification
		Colour	**Pigment**	**Exudate**	**Mycelia**	**Conidia**	**Spore structure**	
Phut	APM-1; APM-15	Colonies green to blue colonies with yellowish shades, velutinous texture	Yes	Yes	Septate	Conidiophores	Hyaline conidiophores smooth-walled stipes, vesicles (6.0µm-17µm wide), metulae (2µm-3µm) and phialides (2µm–4.5µm) in size. Spherical conidia 2.0µm to 3.5µm in diameter, hülle cells present	*Aspergillus*
	APM-6; APM-10	Colonies grown on MEA plates produce blue-green colonies with yellowish shades. Texture is woolly, velutinous (soft, dense, velvety surface)					Sepate hyphae, conidiophores are hyaline, long, and smooth-walled stipes. The vesicles (7.0µm-17µm wide) are clavate (club shaped). Conidiogenous structures are biserate with metulae (2µm-3.5µm) and phialides (2µm–5µm) in size. Conidial structures resemble penicillate (like Penicillium) heads.	*Aspergillus*
	APM-2; APM-3; APM-4; APM-7; APM-9; APM-11; APM-13; APM-14;	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	No	Aseptate	Sporangio-spores	Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor*
	APM-5; APM-8; APM-12;	Whitish colonies on maturity becoming brownish grey, reverse side of colony white in colour	No	No	Aseptate	Sporangio-spores	Rhizoids brownish, opposite the sporangiospore. Sporangiopohores globose and smooth walled becoming dark brown to black brown in colour	*Rhizopus*

Table 8: Macroscopic and microscopic characteristics of filamentous moulds isolated from *dawdim* samples of Mizoram.

Sample	Isolate code	Macroscopic characteristics			Microscopic Characteristics			Tentative identification
		Colour	Pigment	Exudate	Mycelia	Conidia	Spore structure	
Dawdim	MDM-1; MDM-3; MDM-5; MDM-15	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	No	Aseptate	sporangiospore s	Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor circinelloides*
	MDM-2; MDM-6; MDM-7; MDM-18	Colonies diameter of 3-6 cm within 8 days. Colonies are velvety to floccose with yellow green shade	Yes	Yes	Septate	Conidiophores	Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled conidia.	*Penicillium*
	MDM-4; MDM-8; MDM-9; MDM-11, MDM-14; MDM-17	Color of colony is white, slowing turning to grey to blackish after 2-3 days	No	No	Aseptate	Sporangio-spores	Stolon well developed subhyaline to light brown or grayish brown, aseptate, Rhizoids are branched and variable in length. Sporangiophore arising from stolon and directly from aerial hyphae, solitary or 2-3 in groups, straight to slightly curved. Sporangiospores (20-100µm diameter).	*Rhizopus*
	MDM-10; MDM-13; MDM-16; MDM-20	6-7 days colonies show maximum diameter 8.0 mm initially woolly, white, later turn to yellowish, reverse white, or yellow	No	No	Septate	Arthroconidia	Hyphae dichotomously branched, 3-4.5µm diameter, and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.	Not Identified
	MDM-15	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	NO	Aseptate	sporangiospore s	Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor*

Table 9: Macroscopic and microscopic characteristics of filamentous moulds isolated from *khekhrii* samples of Nagaland

Sample	Isolate code	Macroscopic characteristics			Microscopic Characteristics			Tentative identification
		Colour	Pigment	Exudate	Mycelia	Conidia	Spore structure	
Khekhrii	NKM-7;	Colonies diameter of 2-5 cm within 5 days with olive green color.	No	Yes	Septate	Phialosporae	conidiophores upright, simple, aseptate and clavate with swelling. Phialides borne directly on the vesicles and radiating from the entire surface; conidia (phialospores) 1-celled, globose, often variously coloured in mass.	*Aspergillus flavus*
	NKM-8; NKM-12; NKM-13	Colonies show a diameter of 2-5 cm within 5 days, colonies compact white - yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Yes	Yes	Septate	Conidiospore	Septate hyphae straight, prominent with alternatively, unilaterally or oppositely branched. Conidial heads are large (up to 3 mm by 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipeses are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae.	*Aspergillus niger*
	NKM-1; NKM-3; NKM-5; NKM-9; NKM-14	Colonies raise fast, floccose, light yellow to greyish up to 15mm height.	No	No	Aseptate	Sporangio-spores	Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate	*Mucor*
	NKM-2, NKM-4; NKM-6;	Colonies diameter 3-5 cm in 5-6 days, producing dark green conidia with velutinous	Yes	Yes	Septate	Conidiophores	Septate hyphae, conidiophores arising from the substrate, mononematous, usually biverticilate branched. Stipes, smooth walled, branches,	*Penicillium*

Table 9: Macroscopic and microscopic characteristics of filamentous moulds isolated from *khekhrii* samples of Nagaland								
Sample	**Isolate code**	**Macroscopic characteristics**			**Microscopic Characteristics**			**Tentative identification**
		Colour	**Pigment**	**Exudate**	**Mycelia**	**Conidia**	**Spore structure**	
	NKM-10; NKM-11;	colony surface with white edge.					metulae, philalides. Conidia at first sub-globose to ellipsoidal.	
Khekhrii	NKM-15; NKM-16	Colonies on MEA show a diameter of 18–40 mm after 14 days at 25°C. Colonies are flat, radially furrowed with crater-like structure, velvety, powdery to felt-woolly, margins white, aerial mycelium diffuse without prominent exudates but sporulation profuse on all media. Colony surface is olivaceous grey and reverse side is olivaceous black-green	Yes	Yes	Septate	Conidiospore	Mycelium internal and superficial, hyphae sparingly branched, filiform or narrowly cylindrical-oblong, 1−4µm wide, septate, subhyaline or pale olivaceous brown, almost smooth. Conidiophores macro-, semimacro- and micronematous, arising terminally or laterally from hyphae, filiform or narrowly cylindrical branched, 4−110 × 2−3.5µm, 1−7-septate, septa often darkened where ramoconidia secede, but not constricted, subhyaline, pale olivaceous up to pale medium olivaceous brown.	*Cladosporium*

Table 10: Grouping of representative fungal isolates from starters of North East India			
Sample (number of samples)	**Total Number of isolates**	**Representative isolates**	**Isolate code**
Marcha (8)	32	7	SMM-1, SMM-3, SMM-4, SMM-10, SMM-16, SMM-22, SMM-35
Thiat (4)	11	5	MTM-1, MTM-4, MTM-6, MTM-12, MTM-16
Humao (7)	20	6	AEM-1, AEM-3, AEM-4, AEM-8, AXM-1, AMM-3
Hamei (3)	2	20	MHM-1, MHM-15
Chowan (4)	11	5	TCM-1, TCM 4, TCM 7, TCM 9, TCM 12
Phut (6)	15	6	APM-1, APM-3, APM-6, APM-7, APM-12, APM-15
Dawdim (3)	18	6	MDM-1, MDM-10, MDM-11, MDM-14, MDM-16, MDM-18
Khekhrii (5)	16	7	NKM-1, NKM-6, NKM-7, NKM-8, NKM-10, NKM-13, NKM-15

MOLECULAR IDENTIFICATION OF FUNGAL ISOLATES

Genomic DNA of each isolate of 44 filamentous moulds was extracted and PCR products were prepared for identification by ITS gene sequencing. DNA sequences of fungal isolates were assigned by comparing with those available in the GenBank of NCBI database using ITS gene sequence (ITS1 and ITS4) based on *Basic Local Alignment Search Tool* (*BLAST*) 2.0 program (Raja et al. 2017). The phylogenetic trees of nucleotide sequence of 44 filamentous moulds isolated from samples were constructed using the Neighbour-joining method with 1000 replicates bootstrap values (Fig. 11). The ITS gene sequencing results showed three moulds phyla represented by Ascomycota (48%), Mucoromycota (38%) and Basidiomycota (14%) (Fig. 12). Distribution percentage of phyla in starter showed the highest percentage of

Ascomycota (86%) in *khekhrii*, Mucoromycota (60%) in *dawdim* and Basidiomycota (20%) in *chowan, dawdim* and *thiat*, respectively (Fig. 13). Phyla Ascomycota and Mucoromycota were present in all starters, whereas Basidiomycota was present only in *marcha*, *thiat, chowan* and *dawdim.*

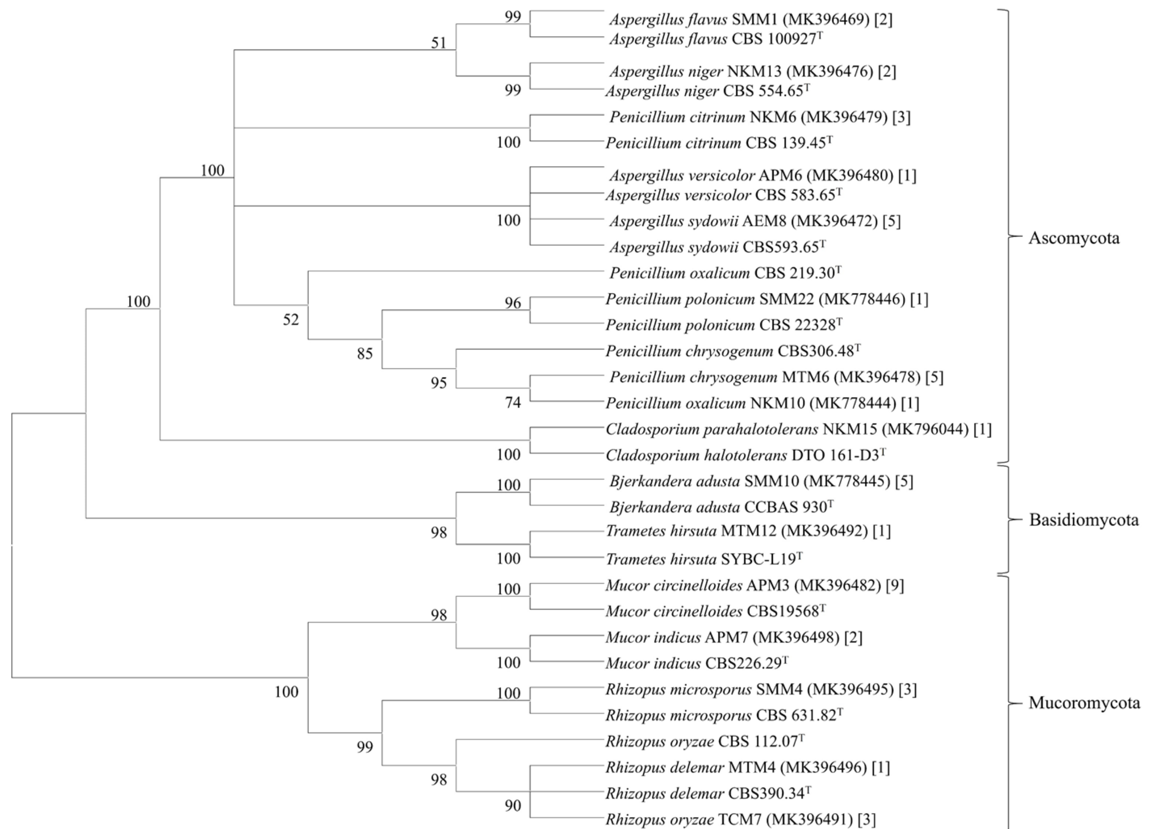

Figure: 11. Molecular phylogenetic analysis of 44 filamentous fungal isolates from starters of North East India using Neighbor-Joining method in MEGA7 software. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branch. The tree is drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The phylogenetic tree branches are collapsed at 50%.

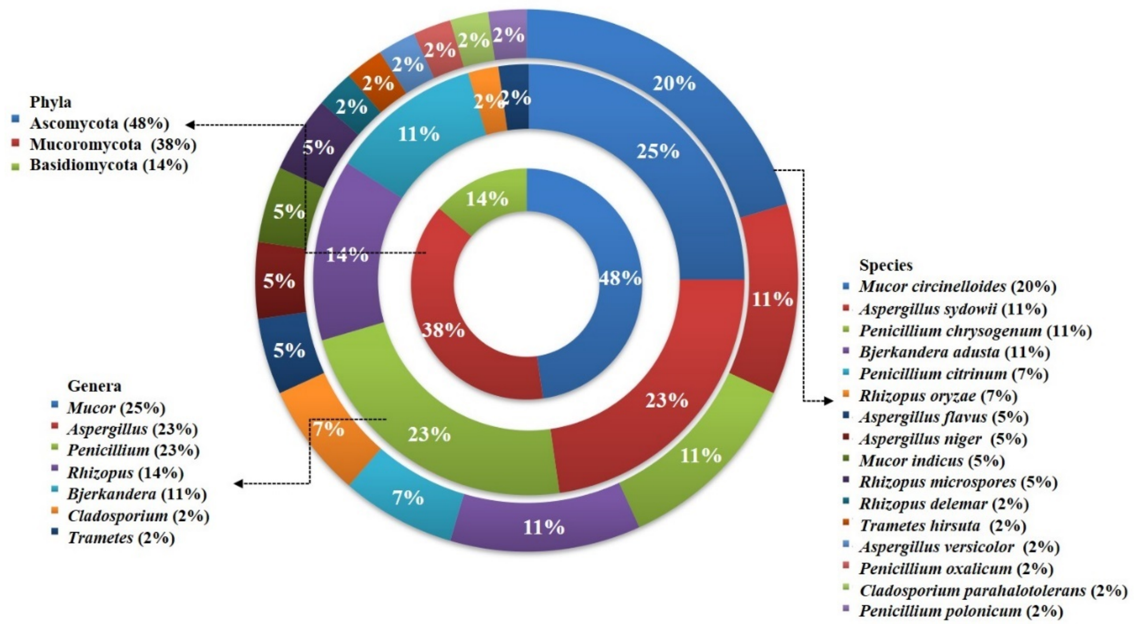

Figure: 12. Abundance percentage distribution of phyla, genus and species the filamentous Moulds isolated from dry starters of North East India.

Based on the results of morphological characteristics and ITS gene sequences, 44 representative strains of filamentous moulds were grouped into 7 genera with 16 species, which were represented by *Mucor circinelloides* (20%), *Aspergillus sydowii* (11%), *Penicillium chrysogenum* (11%), *Bjerkandera adusta* (11%), *Penicillium citrinum* (7%), *Rhizopus oryzae* (7%), *Aspergillus niger* (5%), *Aspergillus flavus* (5%), *Mucor indicus* (5%) *Rhizopus microsporus* (5%), *Rhizopus delemera* (2%), *Aspergillus versicolour* (2%), *Penicillium oxalicum* (2%), *Penicillium polonicum* (2%), *Trametes hirsuta* (2%) and *Cladosporium parahalotolerans* (2%) (Table 11; Fig. 13). Interestingly we detected few basidiomycota filamentous moulds represented by *Bjerkandera adusta* and *Trametes hirsuta* in *marcha, thiat, chowan* and *dawdim* samples (Fig. 14). Colony morphology and microscopic images of 44 species of 7 genera of filamentous moulds isolated from dry starters of India were illustrated for fungal taxonomy in detail sample wise.

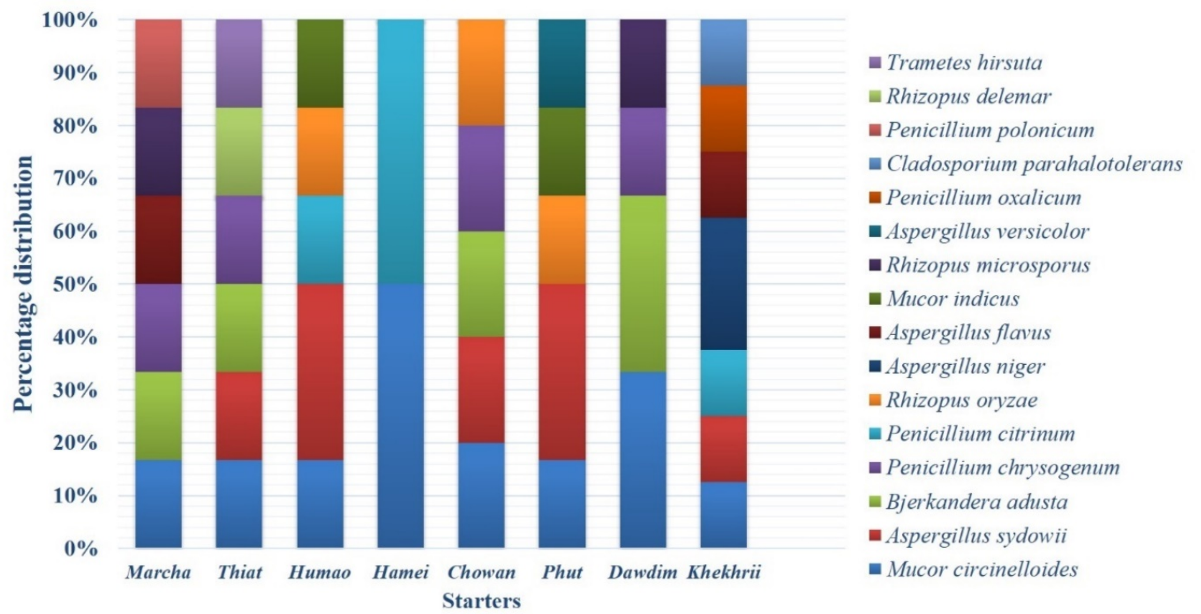

Figure:13. Abundance distribution of the filamentous fungi isolated from dry starters of North East India.

Table: 11. Molecular identification of filamentous moulds isolated from starters of North East India by ITS gene sequence (ITS1 and ITS4) based on BLAST

Product	Isolate code	Identity	GenBank Accession Number	Size in base pair (arbitrary primers)
Marcha	SMM-1	*Aspergillus flavus*	MK396469	519
	SMM-3	*Mucor circinelloides*	MK396489	642
	SMM-4	*Rhizopus microsporus*	MK396495	703
	SMM-10	*Bjerkandera adusta*	MK778445	675
	SMM-16	*Penicillium chrysogenum*	MK396477	577
	SMM-22	*Penicillium polonicum*	MK778446	582
	SMM-35	*Penicillium chrysogenum*	MK778447	552
Thiat	MTM-1	*Mucor circinelloides*	MK396487	636
	MTM-4	*Rhizopus delemar*	MK396496	768
	MTM-6	*Penicillium chrysogenum*	MK396478	583
	MTM-12	*Trametes hirsuta*	MK396492	637
	MTM-16	*Bjerkandera adusta*	MK396500	651
Humao	AEM-1	*Penicillium citrinum*	MK396481	437
	AEM-3	*Rhizopus oryzae*	MK396483	613
	AEM-4	*Mucor circinelloides*	MK396484	648
	AEM-8	*Aspergillus sydowii*	MK396472	467
	AXM-1	*Aspergillus sydowii*	MK396475	546
	AMM-3	*Mucor indicus*	MK778442	565
Hamei	MHM-1	*Mucor circinelloides*	MK796043	601
	MHM-15	*Penicillium citrinum*	MK796042	469
Chowan	TCM-1	*Bjerkandera adusta*	MK396494	520
	TCM-4	*Mucor circinelloides*	MK778449	636
	TCM-7	*Rhizopus oryzae*	MK396491	637
	TCM-9	*Aspergillus sydowii*	MK796041	541
	TCM-12	*Penicillium chrysogenum*	MK778448	541

Table: 11. Molecular identification of filamentous moulds isolated from starters of North East India by ITS gene sequence (ITS1 and ITS4) based on BLAST

Product	Isolate code	Identity	GenBank Accession Number	Size in base pair
Phut	APM-1	*Aspergillus sydowii*	MK396473	577
	APM-3	*Mucor circinelloides*	MK396482	645
	APM-6	*Aspergillus versicolor*	MK396480	417
	APM-7	*Mucor indicus*	MK396498	627
	APM-12	*Rhizopus oryzae*	MK396490	621
	APM-15	*Aspergillus sydowii*	MK396474	574
Dawdim	MDM-1	*Mucor circinelloides*	MK396497	645
	MDM-10	*Bjerkandera adusta*	MK396493	569
	MDM-11	*Rhizopus microsporus*	MK396488	696
	MDM-14	*Mucor circinelloides*	MK396486	641
	MDM-16	*Bjerkandera adusta*	MK396499	680
	MDM-18	*Penicillium chrysogenum*	MK778443	554
Khekhrii	NKM-1	*Mucor circinelloides*	MK796045	490
	NKM-6	*Penicillium citrinum*	MK396479	519
	NKM-7	*Aspergillus flavus*	MK396470	519
	NKM-8	*Aspergillus niger*	MK396471	551
	NKM-10	*Penicillium oxalicum*	MK778444	581
	NKM-13	*Aspergillus niger*	MK396476	602
	NKM-15	*Cladosporium parahalotolerans*	MK796044	546

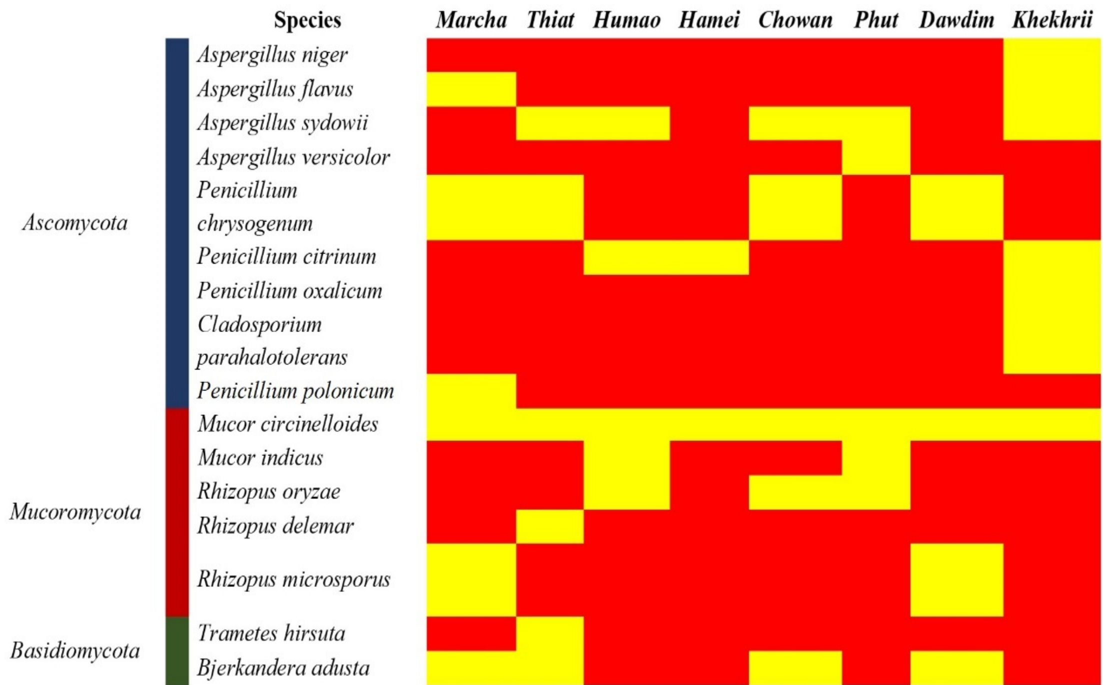

Figure: 14. Heatmap showing the consensus species diversity resulted by ITS-region gene sequencing of filamentous Moulds isolates. We used presence/absence value for fungal species to generate heatmap, where yellow colour indicates the presence and red colour indicates absence.

Frequency and density of fungal species in samples showed that *Aspergillus niger* was colonized with *khekhrii*; species from the *Mucor circinelloides* complex was observed with a high dominance in samples, whereas *Trametes hirsuta* was less diversified and observed only in *thiat* samples (Table 12).

TAXONOMICAL KEYS TO FUNGAL SPECIES

Source: *Marcha* (Sikkim)

Isolate code: SMM-1

Aspergillus flavus Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3 (1): 16 1809.

Macroscopic characteristics: Colonies grown on MEA at 28ºC show a diameter of 2-5 cms within 5 days of incubation with olive green colour (Fig. 15).

Microscopic characteristics: Septate *hyphae* straight, prominent with alternatively, unilaterally or oppositely branched. *Appressoria* long, near to the distal end of the hyphal cells, alternate or unilateral, single-celled, elongate and attenuate at apex, no haustoria. Conidiophores upright, simple, aseptate and clavate with swelling. Phialides borne directly on the vesicles and radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously coloured in mass, and in dry basipetal chains.

Key to Class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed...Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: with inflated apical cells bearing numerous phialides....................

....***Aspergillus***

Key to species:

1) Spore mass: radiate, yellowish green..***A. flavus***

Accession number: MK396469 *Aspergillus flavus* (SMM-1)

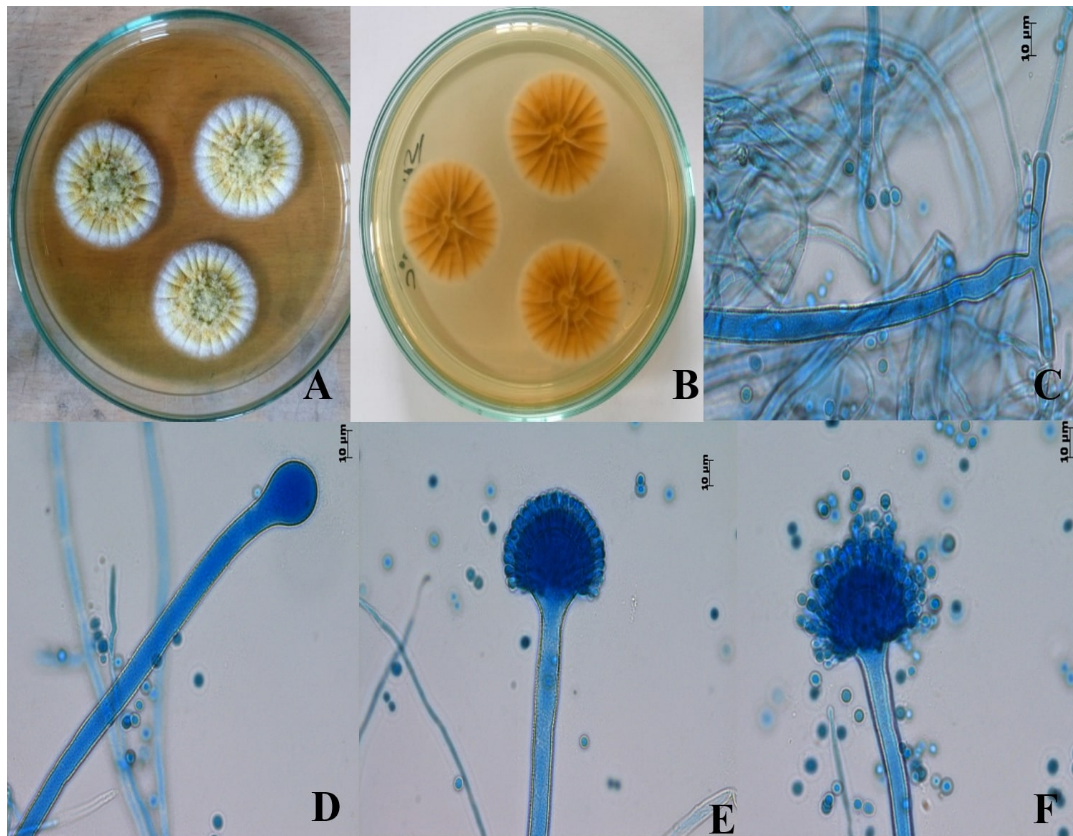

Figure: 15. ***Aspergillus flavus*** (SMM-1) colony on Malt Extract Agar (MEA) surface (A) and reverse (B); (C)septate hyphae with foot cells; (D) conidial head; (E, F) mature globose conidial head contains conidia more typical appearance with phialides radiating from vesicle in all directions. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Marcha* (Sikkim)

Isolate code: SMM-3

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics: Colonies on MEA at 28ºC on the rise fast, floccose, light yellow to greyish up to 15mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 16).

Microscopic morphology: Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted...***Mucor***

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose.............................***M. circinelloides***

Accession number: MK396489 (*M. circinelloides* SMM-3)

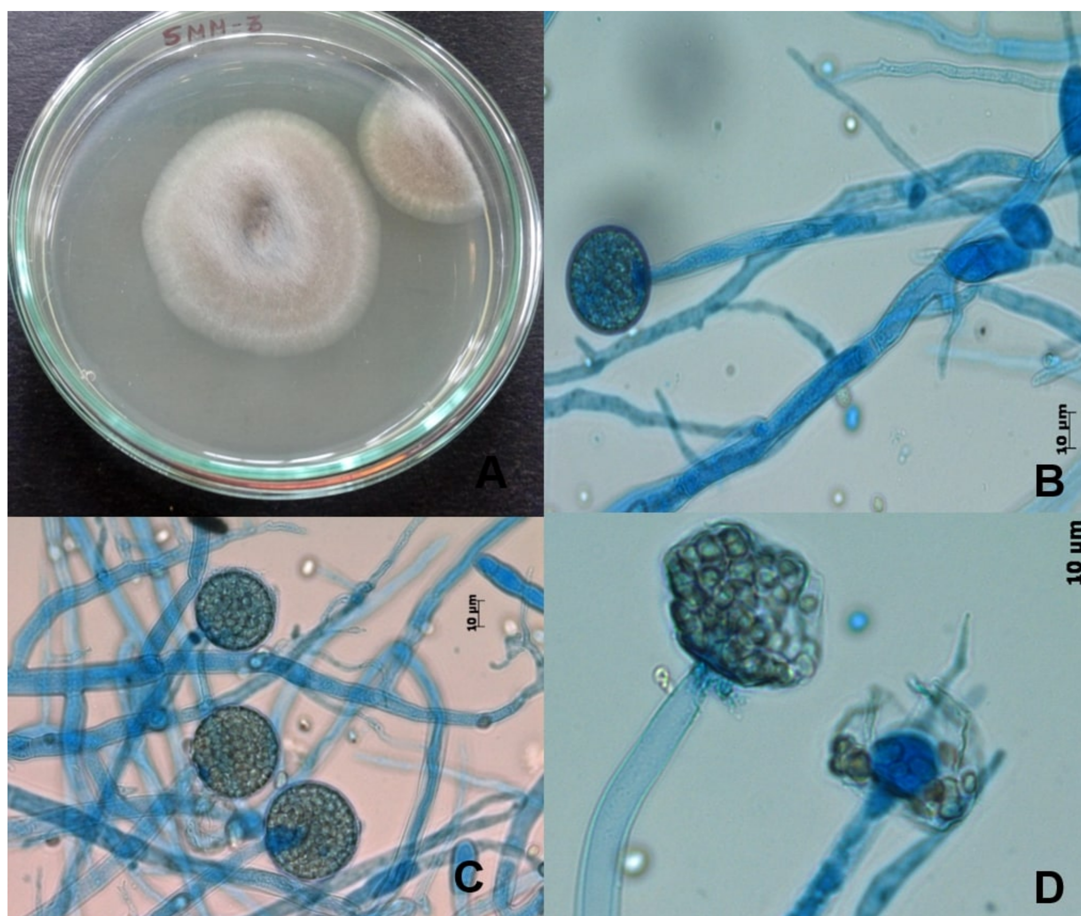

Figure: 16. ***Mucor circinelloides*** (SMM-3) colony on MEA surface (A); (B, C, D) mature globose sporangiophore. Scale bars: A–B = 30 mm, C= 50µm, D–F = 10 µm.

Source: *Marcha* (Sikkim)

Isolate code: SMM-4

Rhizopus microsporus Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 83 (1875).

Macroscopic characteristics: Initially colour of colony on MEA is white, its colour changes slowly to grey to blackish after 2-3 days at 28ºC (Fig. 17).

Microscopic characteristics: Stolon well developed subhyaline to light brown or grayish brown, aseptate, swollen at the point where rhizoids are formed. Rhizoids are branched and variable in length. Sporangiophore arising from stolon and directly from aerial hyphae, solitary or 2-3 in groups, straight to slightly curved. Sporangia globose,

yellowish dark brown when mature. Sporangiophores arising from stolon and opposite to rhizoids, or directly from hyphae. Sporangia globose to depressed globose (20-100µm diameter). Sporangiospores vary or uniform in size and shape, ovoid to sub-globose.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not twisted

6) Rhizoid: formed just below

7) Sporangiophore..***Rhizopus***

Key to species:

1) Sporangiophores arising directly from hyphae. Sporangia globose (20-100µm diameter). Sporangiospores vary or uniform in size and shape, ovoid to sub-globose ....***R. microspores***

Accession number: MK396495 *Rhizopus microsporus* (SMM-4)

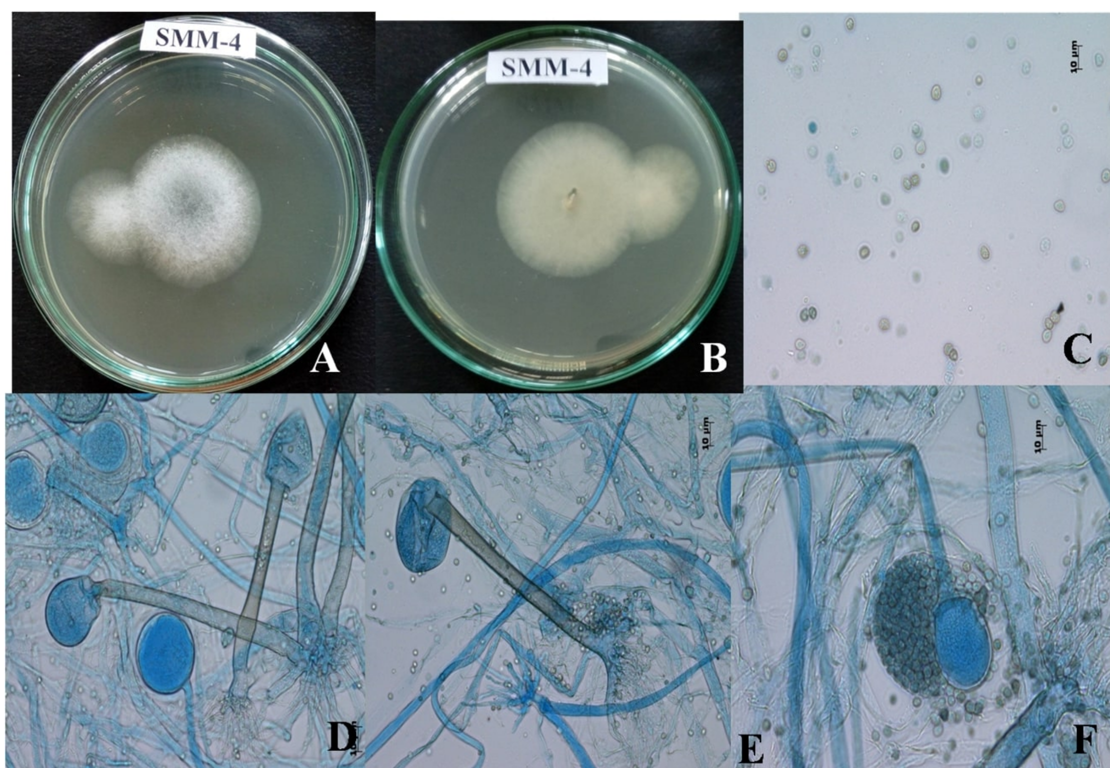

Figure: 17. ***Rhizopus microspores*** (SMM-4) colony pattern on MEA surface (A) and reverse (B); (C)spore structure; (D, E) well-developed stolons, subhyaline light brown aseptate hyphae and sporangiophore with sporangia and rhizoids formed at swollen point; (F) sporangia globose, smooth and released spore. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Marcha* (Sikkim)

Isolate code: SMM-10

Bjerkandera adusta P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 5: 38 (1879).

Macroscopic characteristics: Colonies grown on MEA at 28 C for 6-7 days show maximum growth around 8.0 mm in diameter, aerial mycelium abundant, woolly, initially white, later turn to yellowish, reverse white, or yellow (Fig. 18).

Microscopic characteristics: Hyphae dichotomously branched, 3-4.5µm diameter and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.

Key to class:

1) Hyphae: septate with clamp connection..Basidiomycetes

Key to species:

1) Pores 6–7 per mm, pore surface white (tube mouths) and smoky-gray to grayish-black tinges, dense zone above tubes ....***Bjerkandera adusta***

Accession number: **MK778445** (*Bjerkandera adusta* SMM-10).

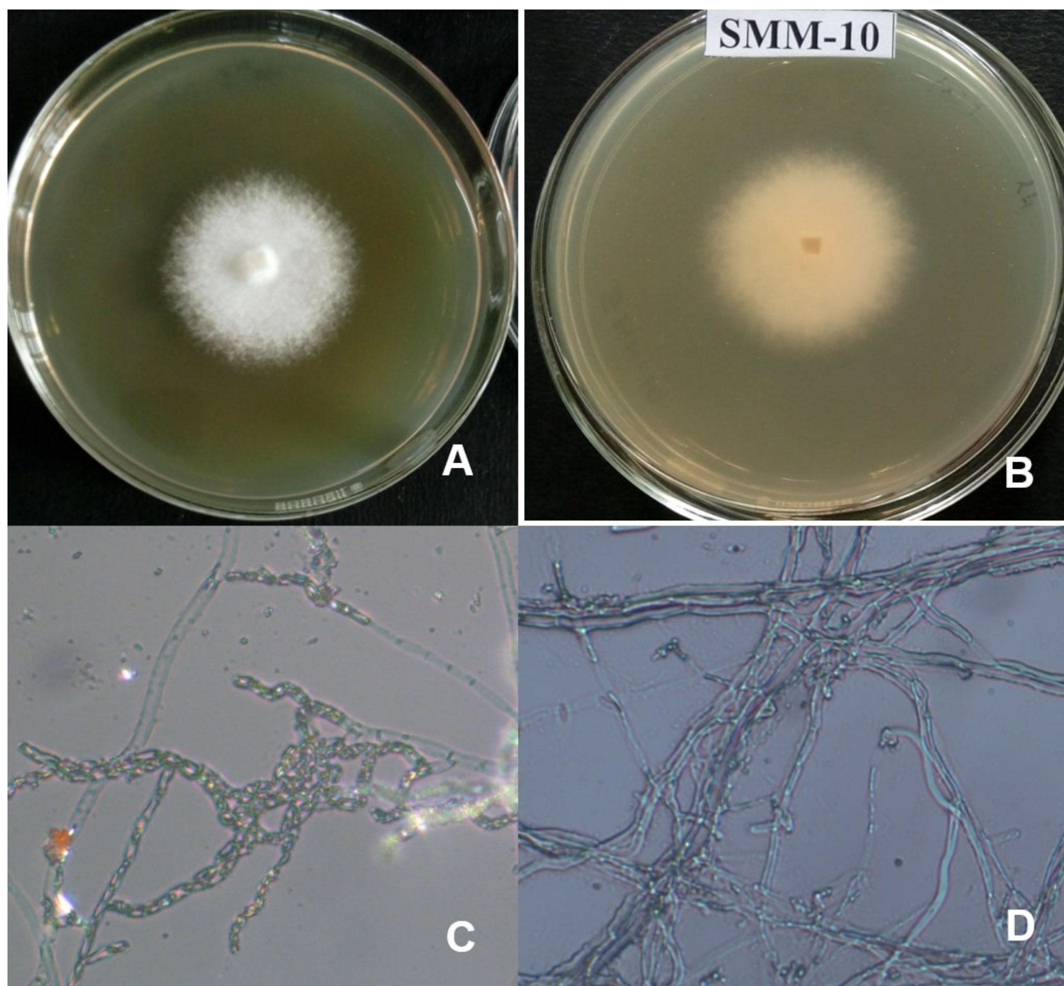

Figure: 18. ***Bjerkandera adusta*** (SMM-10) colony pattern on MEA surface (A) and reverse (B); (C, D) hyphal structure dichotomously branched.

Source: *Marcha* (Sikkim)

Isolate code: SMM-16. SMM-35

Penicillium chrysogenum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 58 (1910).

Macroscopic characteristics: Colonies on MEA grown at 28ºC show a diameter of 3-6 cm within 8 days. Colonies are velvety to floccose with yellow green shade. Exudates typically produce as yellow drops (Fig. 19).

Microscopic characteristics: Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled. Conidia globose, smooth walled.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type....................................Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: without inflated apical cells

3) Conidia: hyaline

4) Conidia: not so globose

5) Conidia: not so boat shaped

6) Conidia: not so clavate

7) Conidia: dry, not cylindrical

8) Conidiophores: well developed

9) Conidiophores: hyaline, spore aggregate in a row

10) Conidiophores: densely penicillate...*Penicillium*

Key to species:

1) Colonies shade green in colour

2) Conidiophores branched

3) Phialides flask shaped conidiophores stipe smooth walled and terverticillate

4) Conidiophores large up to 700-1600µm tall

5) Colonies velvety, often with yellow exudates and reverse conidia globose

....***P. chrysogenum***

Accession number: MK396477 (*Penicillium chrysogenum* SMM-16), **MK778447** (*Penicillium chrysogenum* SMM-35).

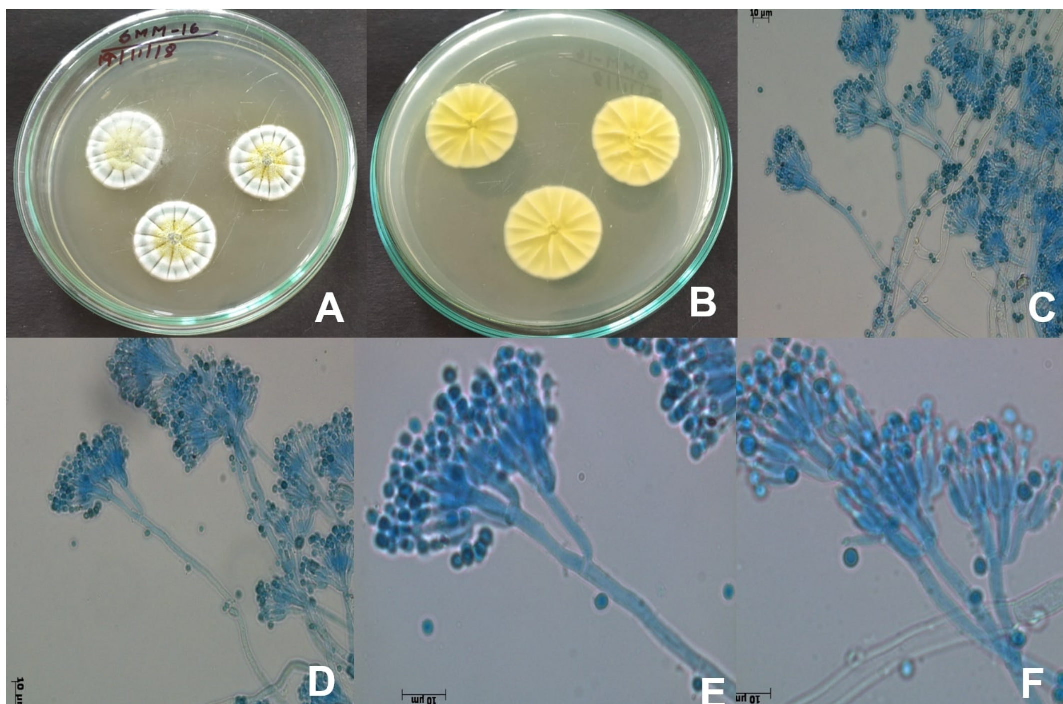

Figure: 19. *Penicillium chrysogenum* (SMM-16; SMM-35) colony on MEA surface (A) and reverse (B); (C, D) septate hyphae with conidiophore; (E, F) mature conidiophores with conidia. Scale bars: A–B = 30 mm, C–F = 10 F.
Source: *Marcha* (Sikkim)

Isolate code: SMM-22

Penicillium polonicum Zalessky, Bulletin International de l'Academie Polonaise des Sciences et des Lettres Série B 1927: 445 (1927).

Macroscopic characteristics: Colonies on MEA at $28^{0}C$ growing moderately producing dull to dark green conidia, velutinous colonies with white edge. Also produce yellow colour exudate droplets and yellow soluble pigments (Fig. 20).

Microscopic characteristics: Septate hyphae, conidiophores arising from the substrate, mononematous, usually terverticilate branched. Stipes, smooth walled, branches, metulate, philalides. Conidia at first sub globose to ellipsoidal.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed

3) Conidia: phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: without inflated apical cells

3) Conidia: hyaline

4) Conidia: not so globose

5) Conidia: not so boat shaped

6) Conidia: not so clavate

7) Conidiophores: well developed

8) Conidia: dry

9) Conidiophores: hyaline, spore aggregate in a row

10) Conidia: not cylindrical

11) Conidia: globose, conidiophores densely penicillate....................*Penicillium*

Key to species:

1) Colonies shade of yellow-green with white

2) Colonies on CYA growing and sporulating well, conidiophores with distinct long stipe and smaller phialides

3) Conidiophores branched

4) Phialides flask shaped conidiophores stipe smooth walled and terverticillate

5) Conidiophores stipe on MEA distinct

6) Conidia smooth, conidiophore stipe rough, conidia smooth to finely rough

7) Conidiophore stipe rough, 2.5-4µm in diameter, colonies fasiculate, reverse pale yellow.

8) Colonies without yellow mycelium and orange brown colour exudates

9) Colonies on MEA at 25ºC in 7 days 8mm in diameter

10) Colonies blue green on MEA, fast rate and good sporulation

11) Conidia globose..*P. polonicum*

Accession number: MK778446 (*Penicillium polonicum* SMM-22)

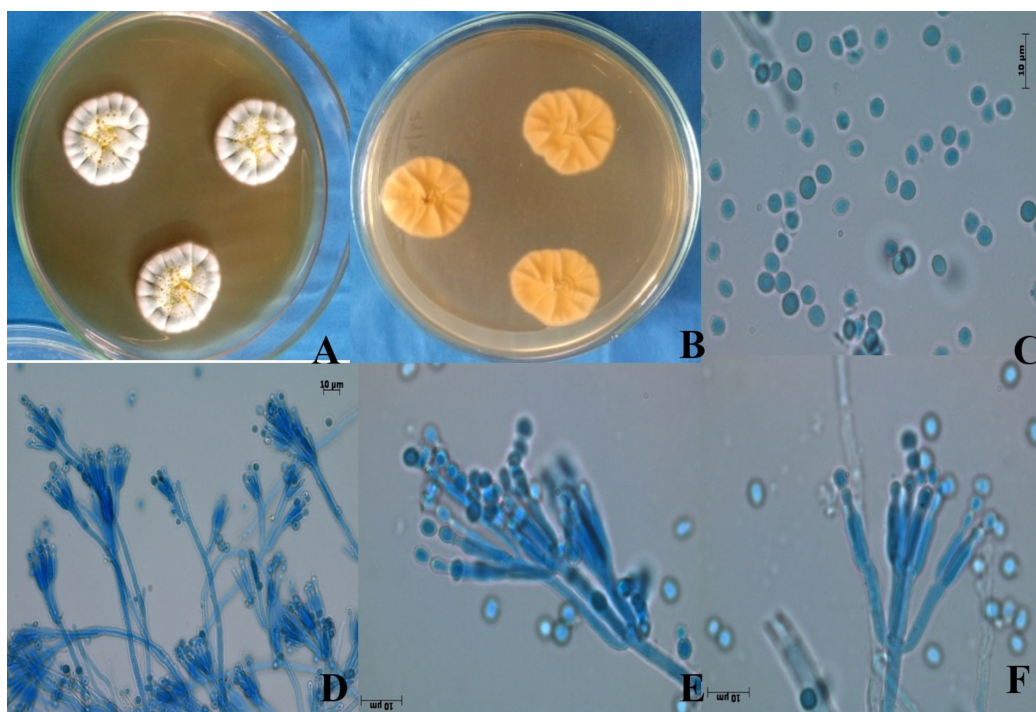

Figure: 20. ***Penicillium polonicum*** (SMM-22) colony on MEA (A) surface and reverse (B); (C) conidial smooth walled and subglobose; (D, E, F) conidiophore was terverticillate, phialides were ampulliform. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Thiat* (Meghalaya)

Isolate code: MTM-1

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics: Colonies on MEA at 28ºC on the rise fast, floccose, light yellow to greyish up to 15 mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 21).

Microscopic characteristics: Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed .... Zygomycetes

Key to zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted .... *Mucor*

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose .... ***M. circinelloides***

Accession number: MK396487 (*Mucor circinelloides* MTM-1)

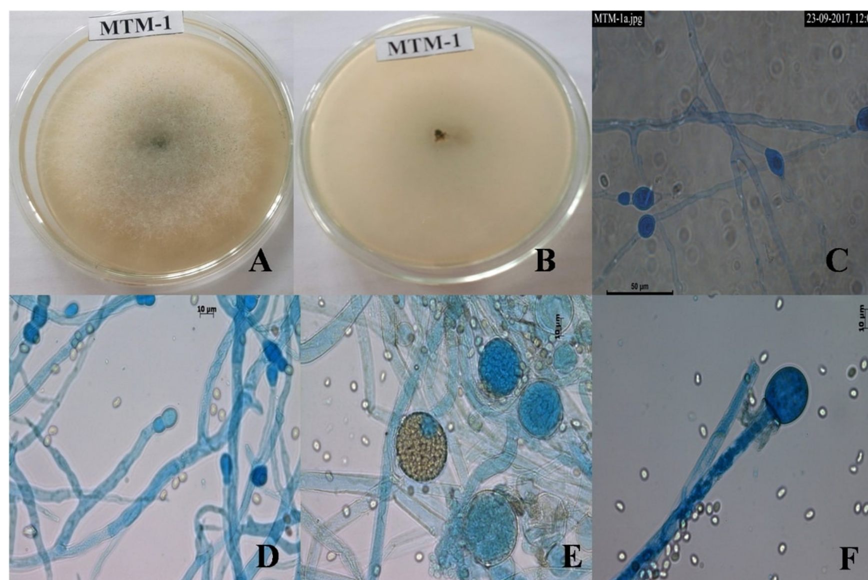

Figure: 21. *Mucor circinelloides* (MTM-1) colony on MEA surface (A) and reverse (B); (C, D) chlamydospore and successive chains of chlamydospore; (E, F) mature globose sporangiospore. Scale bars: A–B = 30 mm, C= 50µm, D–F = 10 µm.

Source: *Thiat* (Meghalaya)

Isolate code: MTM-4

Rhizopus delemar Boidin ex Wehmer and Hanzawa, Mycologisches Centralblatt 1: 86 (1912).

Macroscopic characteristics: Colonies on MEA are white, turning to brownish grey to black when mature, maximum coverage in MEA plate (9 cms diameter) after 2-3 days of incubation at 28ºC (Fig. 22).

Microscopic characteristics: Stolons are well developed, subhyaline to light brown or grayish brown, aseptate, sometimes swollen at the point where rhizoids are formed. Rhizoids are branched, variable in length (very short to comparatively long), grayish brown, paler at the tip. Sporangiophores arising from stolon and opposite rhizoids, or directly from aerial hyphae and not opposite rhizoids, solitary or 2-3 in groups, simple, straight to slightly curved, rarely forked at the apical part smooth Apophyses conspicuous, Sporangia globose (70-170µm), dark brown, without collar. Chlamydospores structure was not observed.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not twisted

6) Rhizoid: formed just below sporangiophore..*Rhizopus*

Key to the species:

Sporangiophores arising from stolon and opposite rhizoids, solitary or 2-3 in groups .... *R. delemar*

Accession number: MK396496 (*Rhizopus delemar* MTM-4).

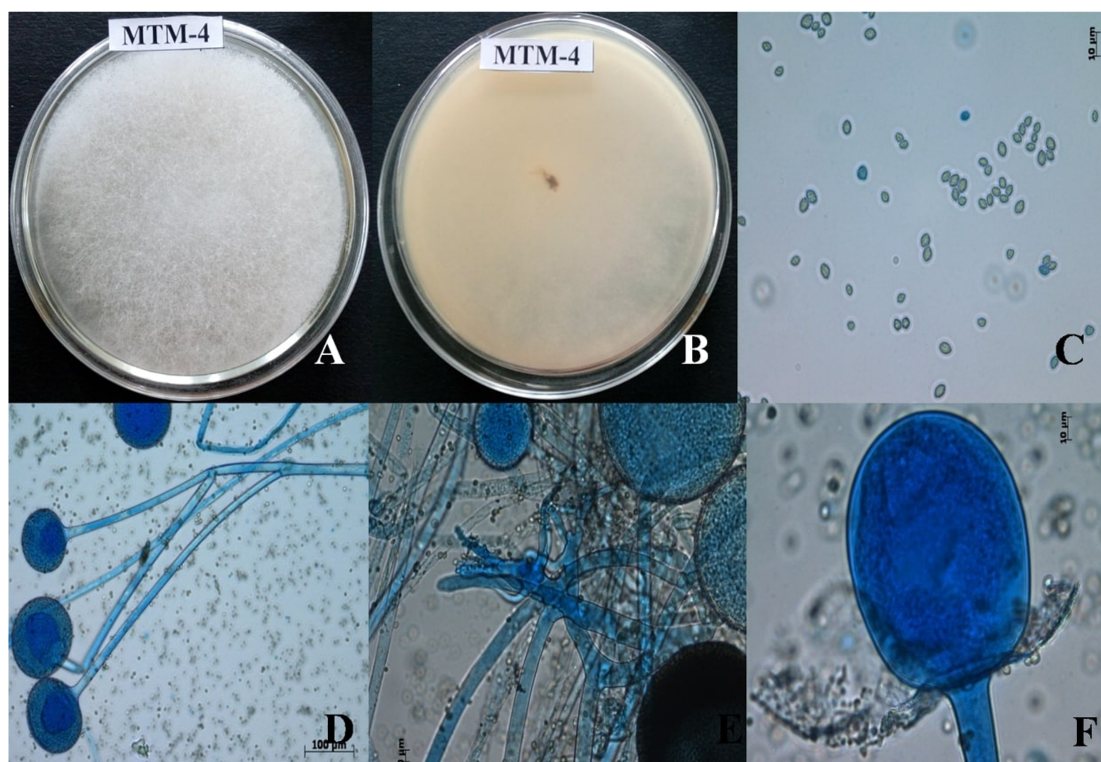

Figure: 22. *Rhizopus delemar* (MTM-4) colony pattern on MEA surface (A) and reverse (B); (C) Oval shape of spores; (D) sporangiophore arising from mycelia with ovoid columellae and distinct apophyses; (E) rhizoid; (F) globose sporangium. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Thiat* (Meghalaya)

Isolate code: MTM-6

Penicillium chrysogenum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 58 (1910).

Macroscopic characteristics: Colonies on MEA grown at 28ºC show a diameter of 3-6 cms within 8 days. Colonies are velvety to floccose with yellow green shade. Exudates typically produce as yellow drops (Fig. 23).

Microscopic characteristics: Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled. Conidia globose, smooth walled.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type...Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: without inflated apical cells

3) Conidia: hyaline

4) Conidia: not so globose

5) Conidia: not so boat shaped

6) Conidia: not so clavate

7) Conidia: dry not cylindrical

8) Conidiophores: well developed

9) Conidiophores: hyaline, spore aggregate in a row

10) Conidiophores: densely penicillate...*Penicillium*

Key to species:

1) Colonies shade green in colour

2) Conidiophores branched

3) Phialides flask shaped conidiophores stipe smooth walled and terverticillate

4) Conidiophores large up to 700-1600µm tall

5) Colonies velvety, often with yellow exudates and reverse conidia globose..........

....***P. chrysogenum***

Accession number: MK396478 (*Penicillium chrysogenum* MTM-6)

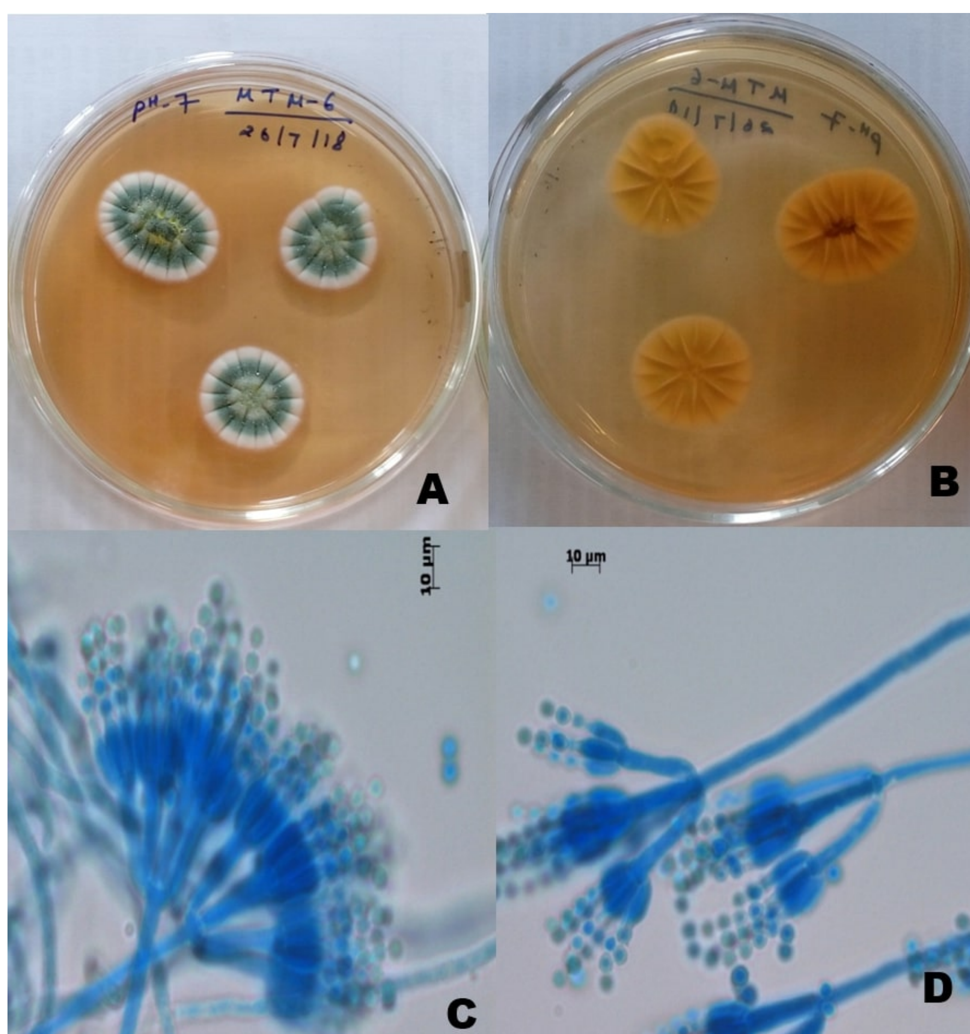

Figure: 23. ***Penicillium chrysogenum*** (MTM-6) colony on MEA surface (A) and reverse (B); (C, D) mature conidiophores with conidia.

Source: T*hiat* (Meghalaya)

Isolate code: MTM-12

Trametes hirsuta (Wulfen) Pilát, Atlas Champ. Eur., Polypor., B: 265 (1939).

Macroscopic characteristics: Colonies on MEA at 28ºC show white cottony mass with no exudation (Fig. 24).

Microscopic characteristics: Thread like mycelium with white and slender hyphae. Hypal system di-trimitic.

Key to class:

1) Hyphae: septate with clamp connection..Basidiomycetes

Key to basidiomycetes:

1) Hyphal system di-trimitic...Trametes

Key to species:

1) Hyphae: trimitic, generative hyphae with clamps, thin walled, skeletal hyphae thick-walled, hyaline, with few branches, binding hyphae thin-walled and brunched..***T. hirsute***

Accession number: MK396492 (*Trametes hirsute* MTM-12).

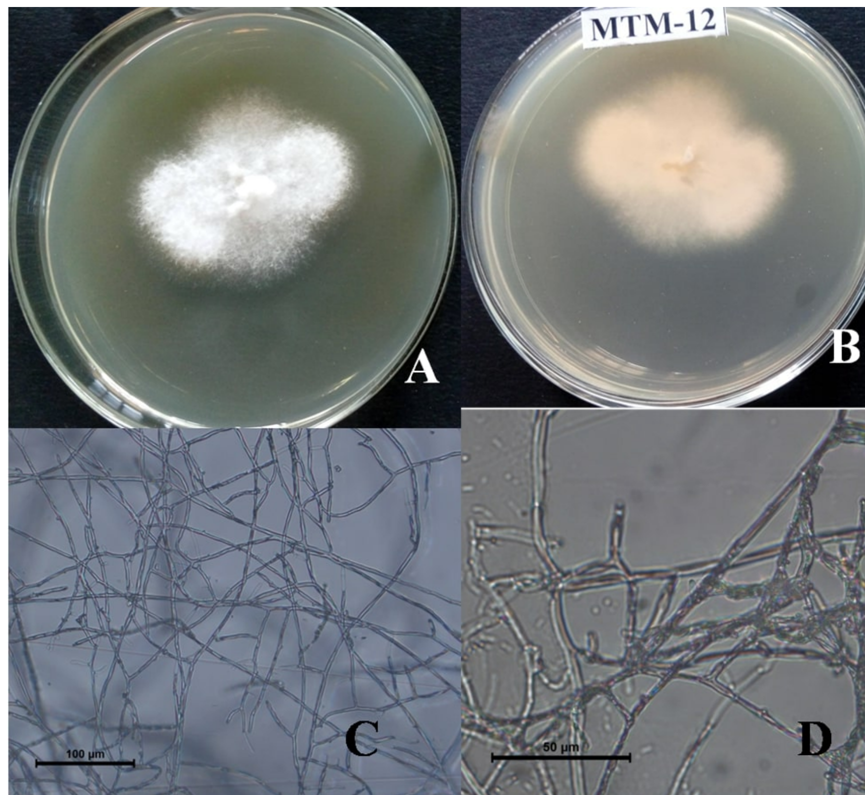

Figure: 24. ***Trametes hirsute*** (MTM-12) colony pattern on MEA surface (A) and reverse (B); (C, D) hyphal structure.

Source: *Thiat* (Meghalaya)

Isolate code: MTM-16

Bjerkandera adusta P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 5: 38 (1879).

Macroscopic characteristics: Colonies grown on MEA at 28 C for 6-7 days show maximum growth around 8.0 mm in diameter, aerial mycelium abundant, woolly, initially white, later turn to yellowish, reverse white, or yellow (Fig. 25).

Microscopic characteristics: Hyphae dichotomously branched, 3-4.5µm diameter and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.

Key to class:

1) Hyphae: septate with clamp connection................................Basidiomycetes

Key to species:

1) Pores 6–7 per mm, pore surface white (tube mouths) and smoky-gray to grayish-black tinges, dense zone above tubes.. ***B. adusta***

Accession number: MK396500 (*Bjerkandera adusta* MTM-16).

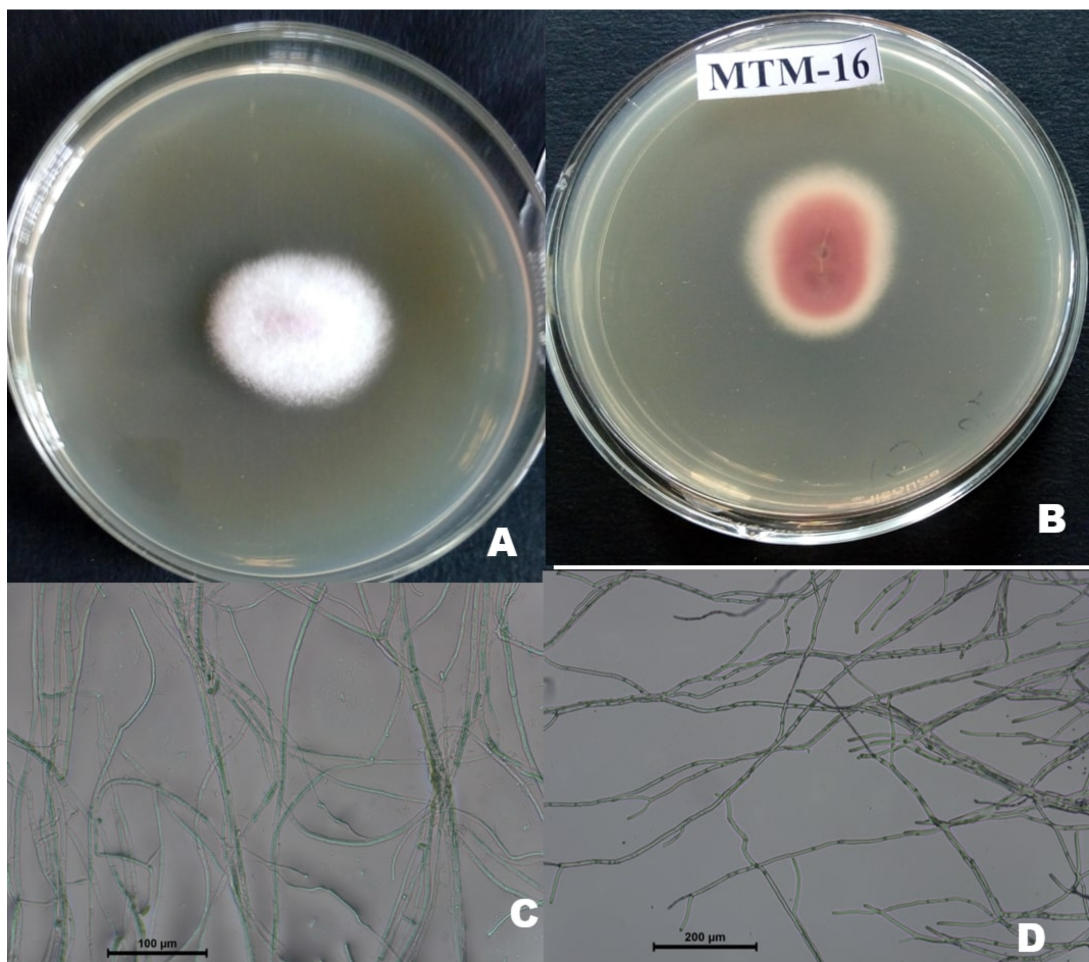

Figure: 25. ***Bjerkandera adusta*** (MTM-16) colony pattern on MEA surface (A) and reverse (B); (C, D) hyphal structure. Scale bars: A–B = 30 mm, C- D= 100-200 µm.

Source: *Humao* (Assam)

Isolate code: AEM-1

Penicillium citrinum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 61 (1910).

Macroscopic characteristics: Colonies on MEA at 28ºC growing restrictedly, attaining a diameter 1-2 cm within 7-8 days, consisting of a dense felt conidiophore with blue green in colour. Reverse colony yellow to orange (Fig. 26).

Microscopic characteristics: Septate hyphae, conidiophores 50-100µm, smooth walled with divergent metulae in a whorl. Metulae bearing 4-5 phialides. Conidia are globose to sub globose, smooth walled, hyaline.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed

3) Conidia: phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidia: hyaline

3) Conidia: not so globose

4) Conidia: not so boat shaped

5) Conidia: not so clavate

6) Conidiophores: well developed

7) Conidia: dry

8) Conidiophores: hyaline, spore aggregate in a row

9) Conidia: not cylindrical

10) Conidia: globose, conidiophores densely penicillate.................*Penicillium*

Key to species:

1) Colonies shade of green

2) Colonies on CYA growing and sporulating well, conidiophores with distinct long stipe and smaller phialides

3) Conidiophores branched

4) Phialides flask shaped conidiophores stipe smooth walled and biverticillate

5) Colonies restricted, diameter less than 1.7cm within 5-6 days on MEA................

....***P. citrinum***

Accession number: MK396481 (*Penicillium citrinum* AEM-1).

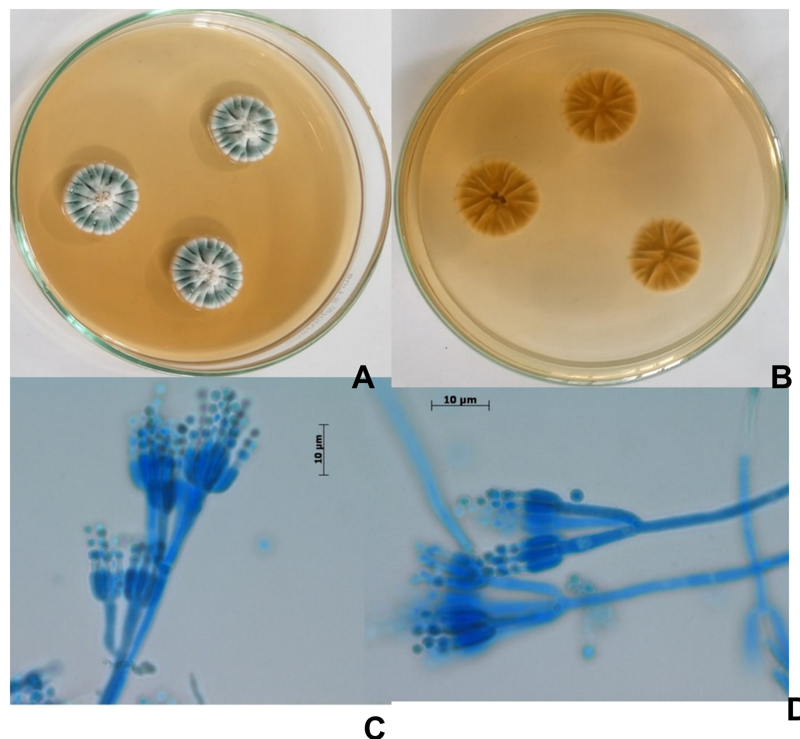

Figure: 26. *Penicillium citrinum* (AEM-1) colony on MEA surface (A) and reverse (B); (C, D) smooth-walled conidiophores stipes (150–280µm) and biverticillate Scale bars: A–B = 30 mm, C–D = 10 µm.

Source: *Humao* (Assam)

Isolate code: AEM-3

Rhizopus oryzae Went & Prinsen Geerlings., Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 4: 16 (1895).

Macroscopic characteristics: Colonies on MEA at 28^0C whitish becoming brownish grey with maturity and reverse side of colony white in colour (Fig. 27).

Microscopic characteristics: Aseptate hyphae with rhizoids brownish, opposite the sporangiospore. Sporangiopohore globose and smooth walled becoming dark brown to black brown in colour.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not twisted

6) Rhizoid: formed just below sporangiophore.. ....*Rhizopus*

Key to species:

1) Sporangiophore striate. Sporangiophore variable in length, up to 4mm long Growth at 37ºC stolon with chlamydospore. Sporangiophores mostly 1-1.5mm long............ ....***R. oryzae***

Accession number: MK396483 (*Rhizopus oryzae* AEM-3)

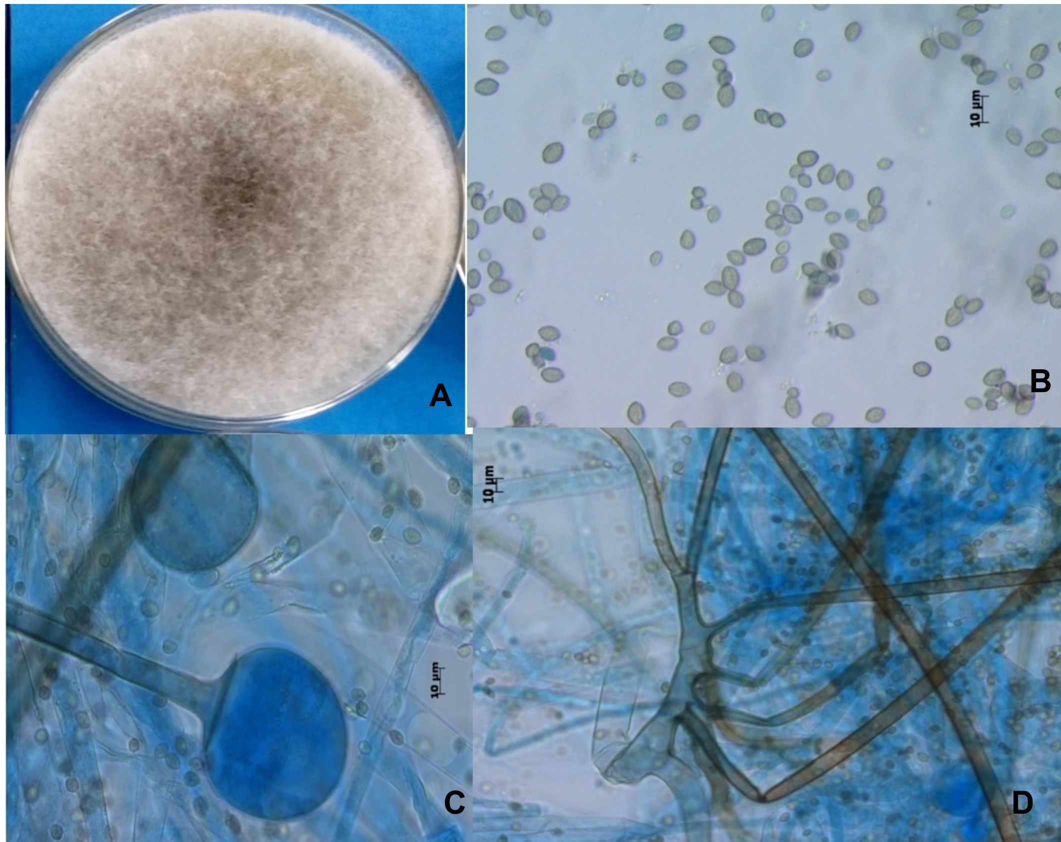

Figure: 27. ***Rhizopus oryzae*** (AEM-3) colony pattern on MEA surface (A) and Spore structure (B); (C)sporangiospores; (D) Branched rhizoids usually in groups. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Humao* (Assam)

Isolate code: AEM-4

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics: Colonies on MEA at 28ºC on the rise fast, floccose, light yellow to greyish up to 15 mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 28).

Microscopic characteristics: Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella ovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to zygomycetes:

1) vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted..*Mucor*

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose..............................***M. circinelloides***

Accession number: MK396484 (*Mucor circinelloides* AEM-4)

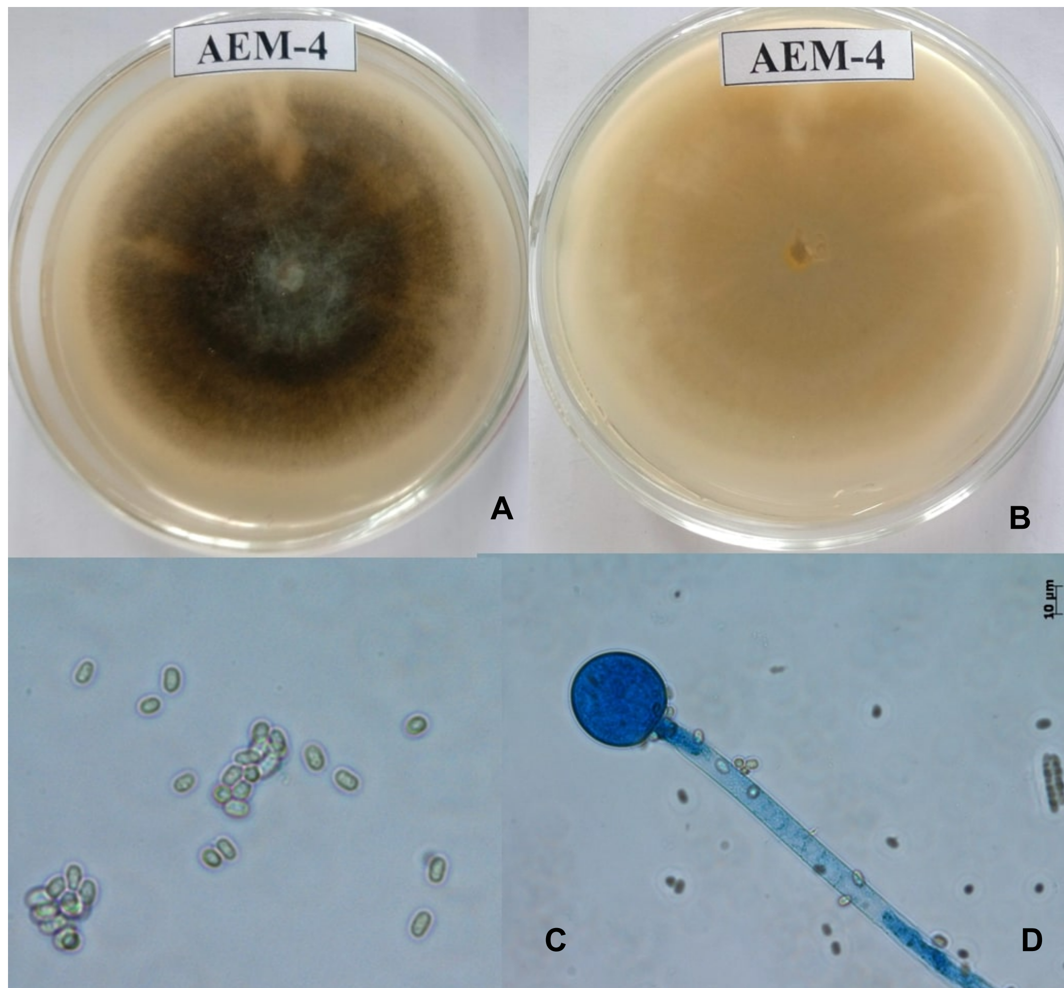

Figure: 28. ***Mucor circinelloides*** (AEM-4) colony on MEA surface (A) and reverse (B); (C) spores; (D) mature globose sporangiospore. Scale bars: A–B = 30 mm, C= 50µm, D–F = 10 µm.

Source: *Humao* (Assam)

Isolate code: AEM-8, AXM-1

Aspergillus sydowii Thom & Church, The Aspergilli: 147 (1926)

Macroscopic characteristics: Colonies grown on MEA plates produce blue-green colonies with yellowish shades. Texture is woolly, velutinous (soft, dense, velvety surface) (Fig. 29).

Microscopic characteristics: Conidiophores are hyaline, long and smooth-walled stipes. The vesicles (7.0µm-17µm wide) are clavate (club shaped). Conidiogenous structures are biserate with metulae (2µm-3.5µm) and phialides (2µm–5µm) in size.

Conidial structures resemble penicillate (like Penicillium) heads. Conidia are spherical (2.5µm to 3.5µm in diameter). Presence of hülle cells which are covering cleisthothecia.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type....................................Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: with inflated apical cells bearing numerous phialides......................

....*Aspergillus*

Key to species:

1) Conidial heads biseriate, colonies "Deflet blue green"...

....***A. sydowii***

Accession number: MK396472 (*Aspergillus sydowii* AEM-8), **MK396475** (*Aspergillus sydowii* AXM-1).

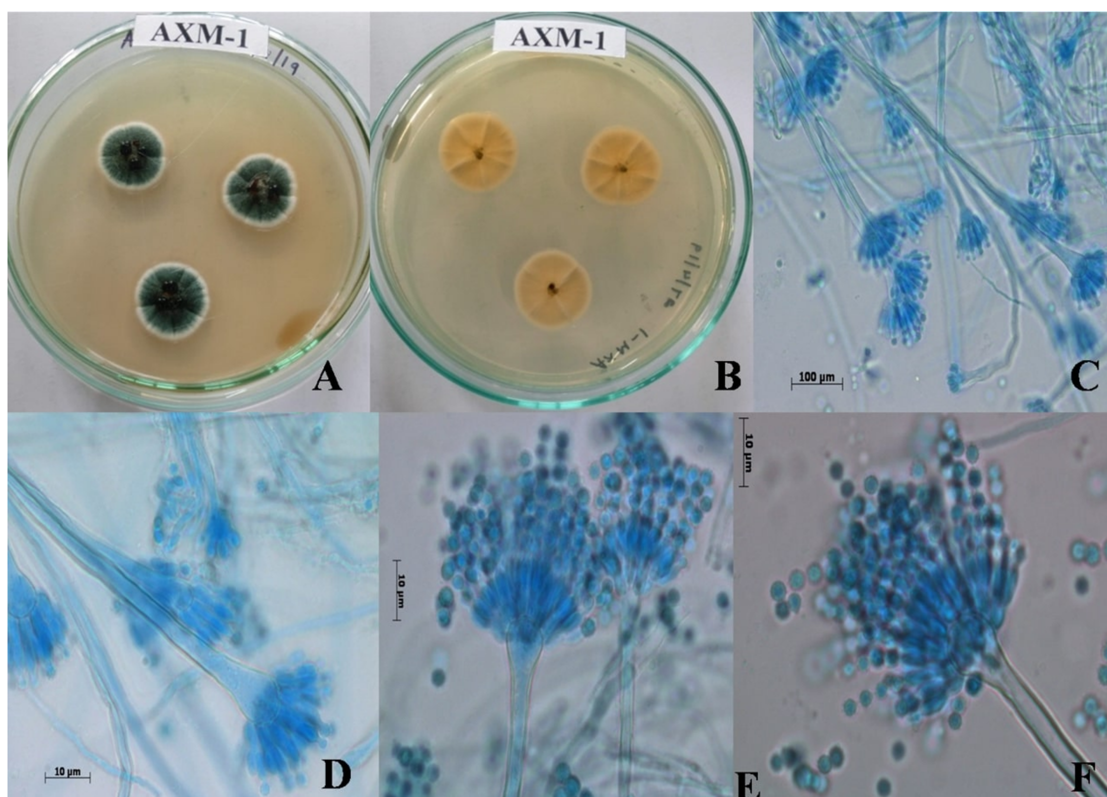

Figure: 29. ***Aspergillus sydowii*** (AEM-8; AXM-1) colony on MEA surface (A) and reverse (B); (C, D, E, F) immature conidiophore with vesicle bearing conidiogenous metulae and phialides (biserate). Scale bars: A–B = 30 mm, C=100µM, D–F = 10 µm.

Source: *Humao* (Assam)

Isolate code: AMM-3

Mucor indicus Lendn., Bulletin de la Société Botanique de Genève 21: 258 (1930).

Macroscopic characteristics: Colonies on MEA at 28^{0}C are growing fast, cottony to fluffy, white to yellow, becoming dark-grey, with the development of sporangia (Fig. 30).

Microscopic characteristics: Sporangiophores are erect, simple or branched, forming large (60-300µm in diameter), terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae. A conspicuous collarette (remnants of the sporangial wall) is usually visible at the base of the columella after sporangiospore dispersal. Sporangiospores are hyaline, grey or brownish, globose

to ellipsoidal and smooth-walled or finely ornamented. Chlamydospores and zygospores also present, mycelia aseptate.

Key to class

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted..*Mucor*

Key to the species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose....................................*M. indicus*

Accession number: MK778442 (*Mucor indicus* AMM-3)

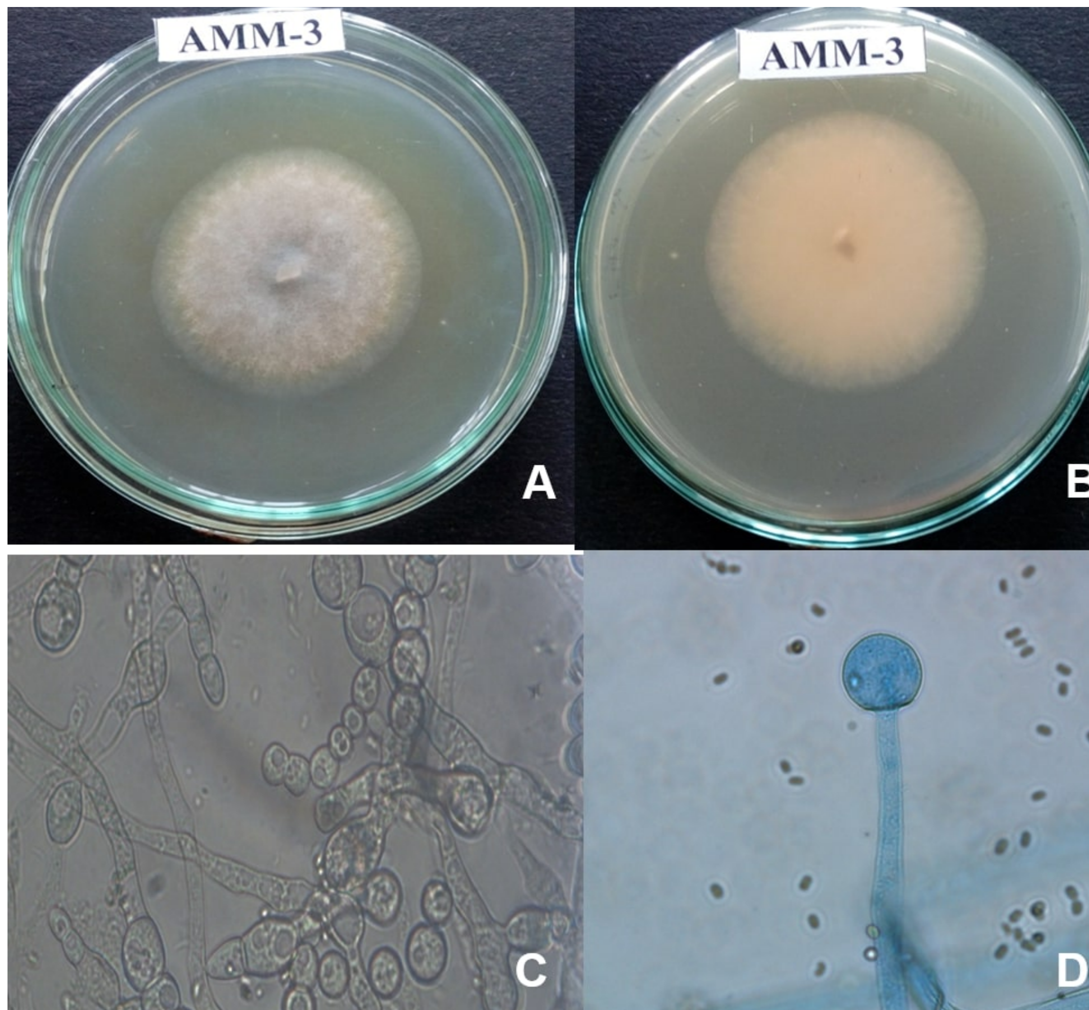

Figure: 30. ***Mucor indicus*** (AMM-3) colony pattern on MEA surface (A) and reverse (B); (C)hyphae with chains of chlamydospore (D)mature sporangiospores contain sporangiospores. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Hamei* (Manipur)

Isolate code: MHM-1

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics: Colonies on MEA at 28ºC on the rise fast, floccose, light yellow to greyish up to 15mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 31).

Microscopic characteristics: Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella ovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted..*Mucor*

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose...............................***M. circinelloides***

Accession number: MK796043 (*Mucor circinelloides* MHM-1)

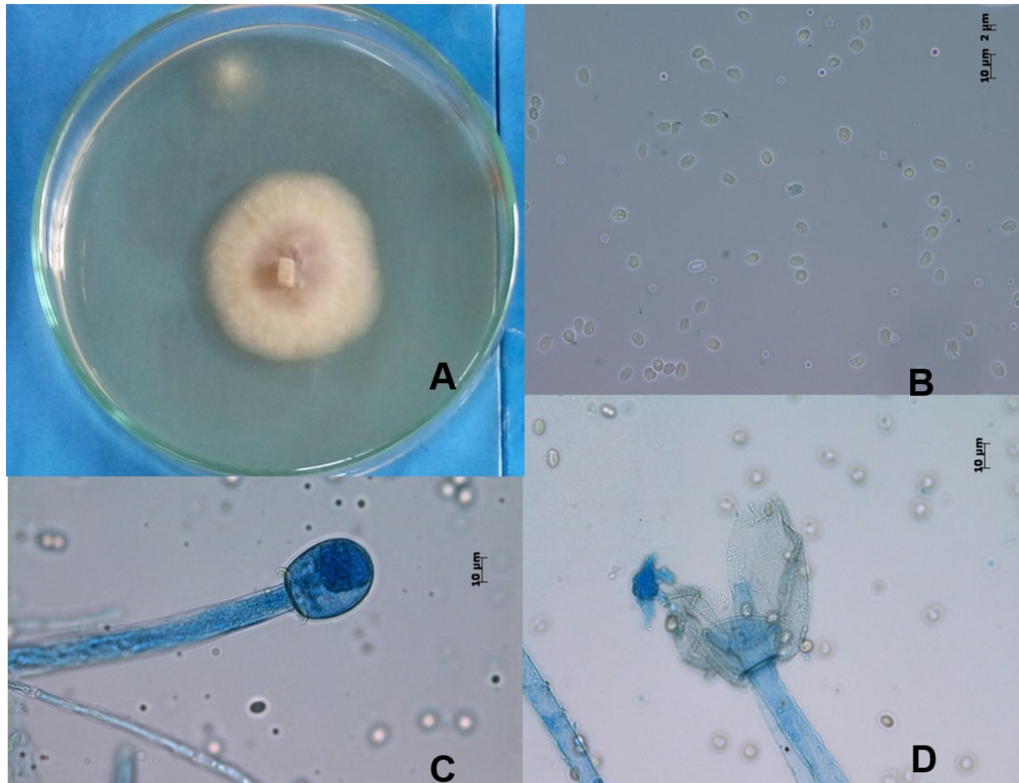

Figure: 31. ***Mucor circinelloides*** (MHM-1) colony on MEA surface (A) and (B) spores; (C, D) mature oval sporangiospore. Scale bars: A = 30 mm, C-D = 10 µm.

Source: *Hamei* (Manipur)

Isolate code: MHM-15

Penicillium citrinum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 61 (1910).

Macroscopic characteristics: Colonies on MEA at 28ºC were growing restrictedly, attaining a diameter 1-2 cm within 7-8 days, consisting of a dense felt conidiophore with blue green in colour. Reverse colony changes its colour from yellow to orange (Fig. 32).

Microscopic characteristics: Septate hyphae, conidiophores 50-100µm, smooth walled with divergent metulae in a whorl. Metulae bearing 4-5 phialides. Conidia are globose to sub globose, smooth walled, hyaline.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed

3) Conidia: phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidia: hyaline

3) Conidia: not so globose

4) Conidia: not so boat shaped

5) Conidia: not so clavate

6) Conidiophores: well developed

7) Conidia: dry

8) Conidiophores: hyaline, spore aggregate in a row

9) Conidia: not cylindrical

10) Conidia: globose, conidiophores densely penicillate..................*Penicillium*

Key to species:

1) Colonies shade of green

2) Colonies on CYA growing and sporulating well, conidiophores with distinct long stipe and smaller phialides

3) Conidiophores branched

4) Phialides flask shaped conidiophores stipe smooth walled and biverticillate

5) Colonies restricted, diameter less than 1.7cm within 5-6 days on MEA.. ***P. citrinum***

Accession number: MK796042 (*Penicillium citrinum* MHM-15).

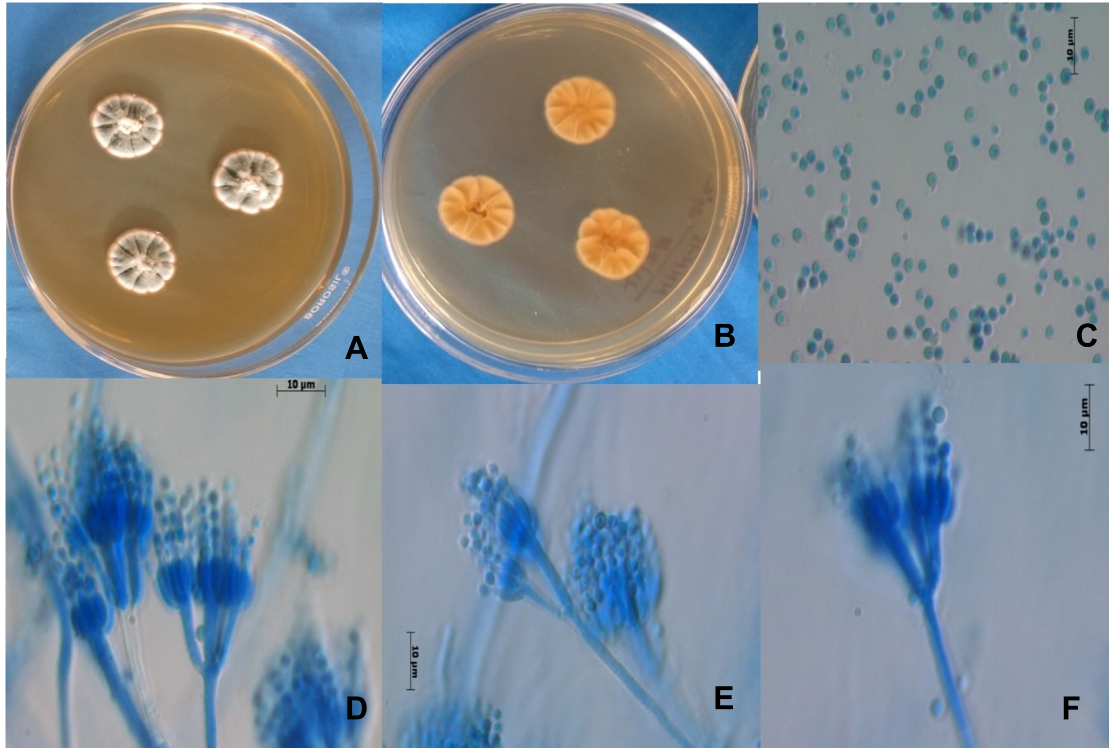

Figure: 32. *Penicillium citrinum* (MHM-15) colony on MEA surface (A) and reverse (B); (C) smooth-walled conidiophores stipes (150–280µm) and biverticillate (D,E,F) phialides ampuliform (flask-shaped) and about 8–12 µm in length, conidia (2.2–3.0 µm diameter) globose to sub-globose. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Chowan* (Tripura)

Isolate code: TCM-1

Bjerkandera adusta P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 5: 38 (1879).

Macroscopic characteristics: Colonies grown on MEA at 28 C for 6-7 days show maximum growth around 8.0 mm in diameter, aerial mycelium abundant, woolly, initially white, later turn to yellowish, reverse white, or yellow (Fig. 33).

Microscopic characteristics: Hyphae dichotomously branched, 3-4.5µm diameter, and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.

Key to class:

1) Hyphae: septate with clamp connection.............................Basidiomycetes

Key to species:

1) Pores 6–7 per mm, pore surface white (tube mouths) and smoky-gray to grayish-black tinges, dense zone above tubes..***B. adusta***

Accession number: MK396494 (*Bjerkandera adusta* TCM-1).

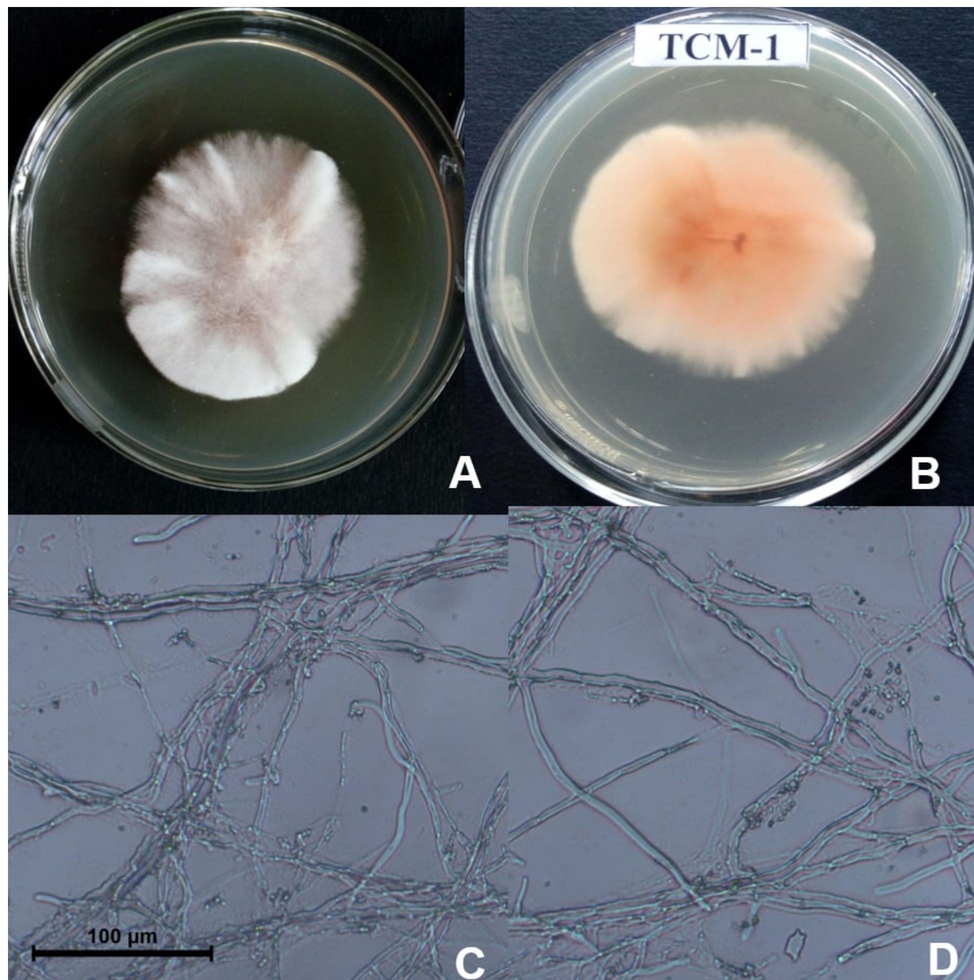

Figure: 33. ***Bjerkandera adusta*** (TCM-1) colony pattern on MEA surface (A) and reverse (B); (C, D) hyphal structure dichotomously branched.

Source: *Chowan* (Tripura)

Isolate code: TCM-4

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics: Colonies on MEA at 28ºC on the rise, floccose, light yellow to greyish up to 15mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 34).

Microscopic characteristics: Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella ovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted..*Mucor*

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant Sporangiospores pigmented, sub-globose..................

....***M. circinelloides***

Accession number: **MK778449** (*Mucor circinelloides* TCM-4)

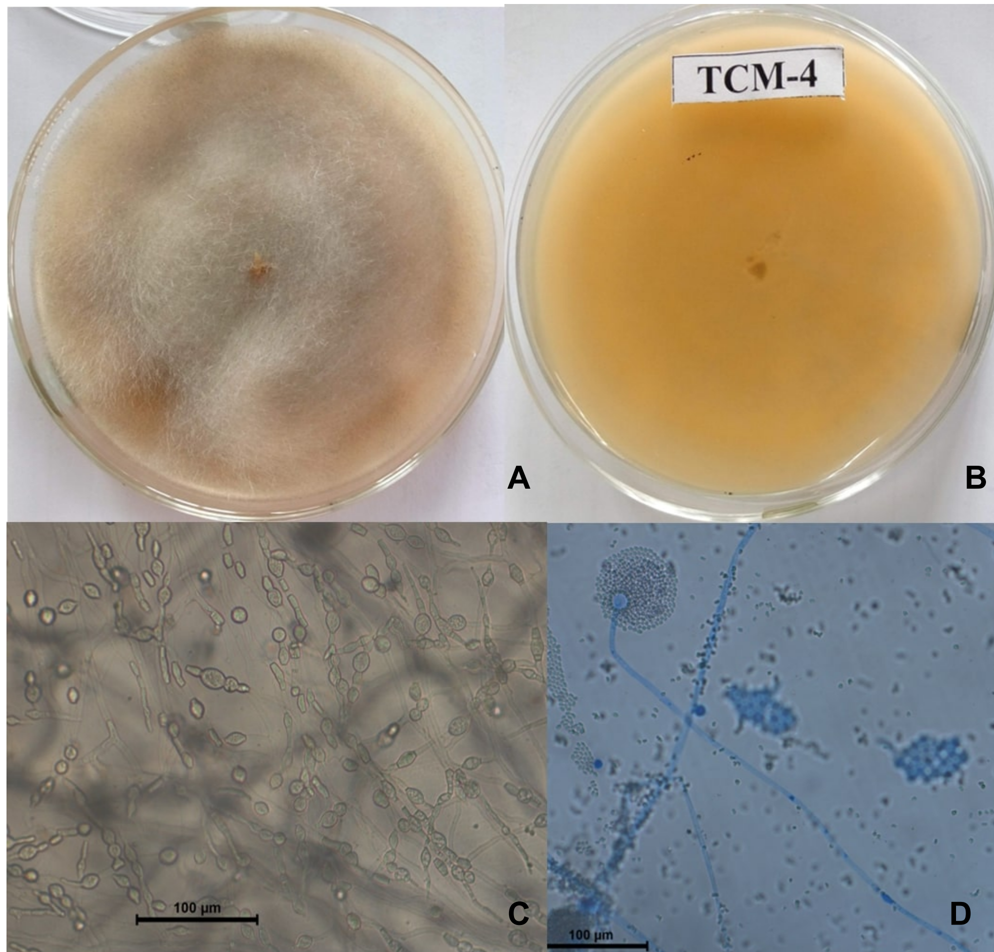

Figure: 34. ***Mucor circinelloides*** (TCM-4) colony on MEA surface (A) and (B) spores; (C) Chlamydospore (D) mature sporangiospore. Scale bars: A = 30 mm, C-D = 100 µm.

Source: *Chowan* (Tripura),

Isolate code: TCM-7

Rhizopus oryzae Went & Prinsen Geerlings., Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 4: 16 (1895).

Macroscopic characteristics: Colonies on MEA at 28^0C whitish becoming brownish grey with maturity and reverse side of colony white in colour (Fig. 35).

Microscopic characteristics: Aseptate hyphae with rhizoids brownish, opposite the sporangiospore. Sporangiophore globose and smooth walled becoming dark brown to black brown in colour.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not twisted

6) Rhizoid: formed just below Sporangiophore..

....*Rhizopus*

Key to species:

1) Sporangiophore striate. Sporangiophore variable in length, up to 4mm long

Growth at 37ºC stolon with chlamydospore. Sporangiophores mostly 1-1.5mm long

....***R. oryzae***

Accession number: MK396491 (*Rhizopus oryzae* TCM-7).

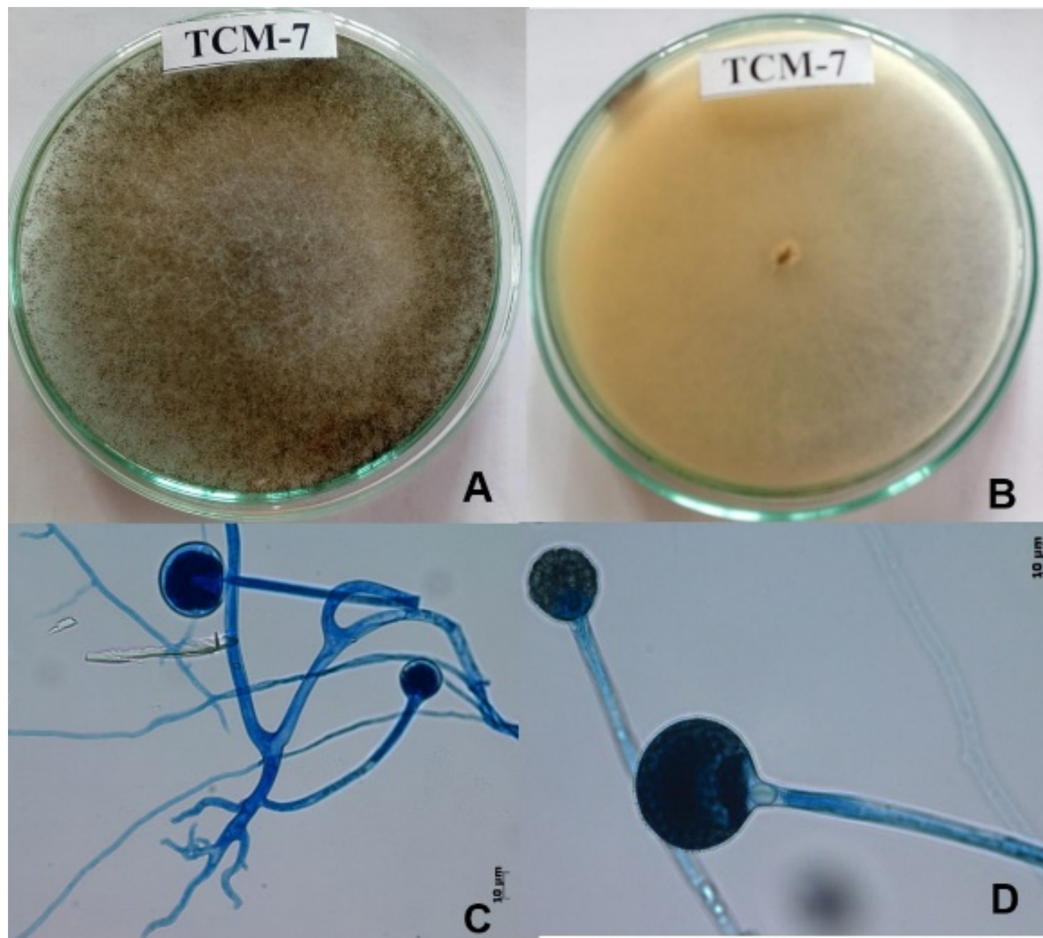

Figure: 35. ***Rhizopus oryzae*** (TCM-7) colony pattern on MEA surface (A) and Spore structure (B); (C)sporangiospores; (D) Branched rhizoids usually in groups. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Chowan* (Tripura)

Isolate code: TCM-9

Aspergillus sydowii Thom & Church, The Aspergilli: 147 (1926)

Macroscopic characteristics: Colonies grown on MEA plates produce blue-green colonies with yellowish shades. Texture is woolly, velutinous (soft, dense and velvety surface) (Fig. 36).

Microscopic characteristics: Conidiophores are hyaline, long, and smooth-walled stipes. The vesicles (7.0µm-17µm wide) are clavate (club shaped). Conidiogenous structures are biserate with metulae (2µm-3.5µm) and phialides (2µm–5µm) in size.

Conidial structures resemble penicillate (like Penicillium) heads. Conidia are spherical (2.5µm to 3.5µm in diameter). Presence of hülle cells which are covering cleisthothecia.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3)Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type.................................Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: with inflated apical cells bearing numerous phialides.......................

....*Aspergillus*

Key to species:

1) Conidial heads biseriate, colonies "Deflet blue green"...

....***A. sydowii***

Accession number: MK796041 (*Aspergillus sydowii* TCM-9)

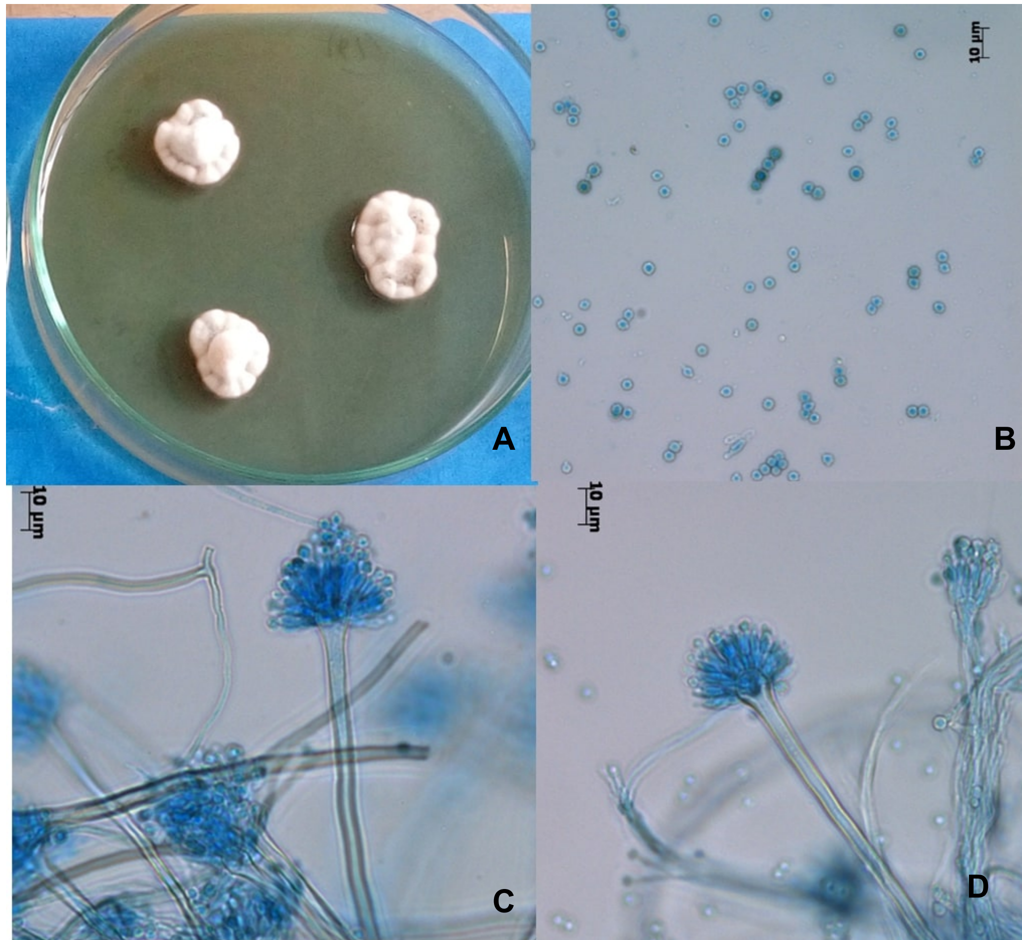

Figure: 36. ***Aspergillus sydowii*** (TCM-9) colony on MEA surface (A) (B) Spore (C, D) immature conidiophore with vesicle bearing conidiogenous metulae and phialides Scale bars: A–B = 30 mm, C- D = 10 µm.

Source: *Chowan* (Tripura)

Isolate code: TCM-12

Penicillium chrysogenum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 58 (1910).

Macroscopic characteristics: Colonies on MEA grown at 28ºC show a diameter of 3-6 cms within 8 days. Colonies are velvety to floccose with yellow green shade. Exudates typically produce as yellow drops (Fig. 37).

Microscopic characteristics: Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides.

Phialides were flask shaped, often with thick walled. Conidia globose with smooth walled.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed...Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type...Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: without inflated apical cells

3) Conidia: hyaline

4) Conidia: not so globose

5) Conidia: not so boat shaped

6) Conidia: not so clavate

7) Conidia: dry, not cylindrical

8) Conidiophores: well developed

9) Conidiophores: hyaline, spore aggregate in a row

10) Conidiophores: densely penicillate..*Penicillium*

Key to species:

1) Colonies shade green in colour

2) Conidiophores branched

3) Phialides flask shaped conidiophores stipe smooth walled and terverticillate

4) Conidiophores large up to 700-1600µm tall

5) Colonies velvety, often with yellow exudates and reverse conidia globose..........

....***P. chrysogenum***

Accession number: MK778448 (*Penicillium chrysogenum* TCM-12)

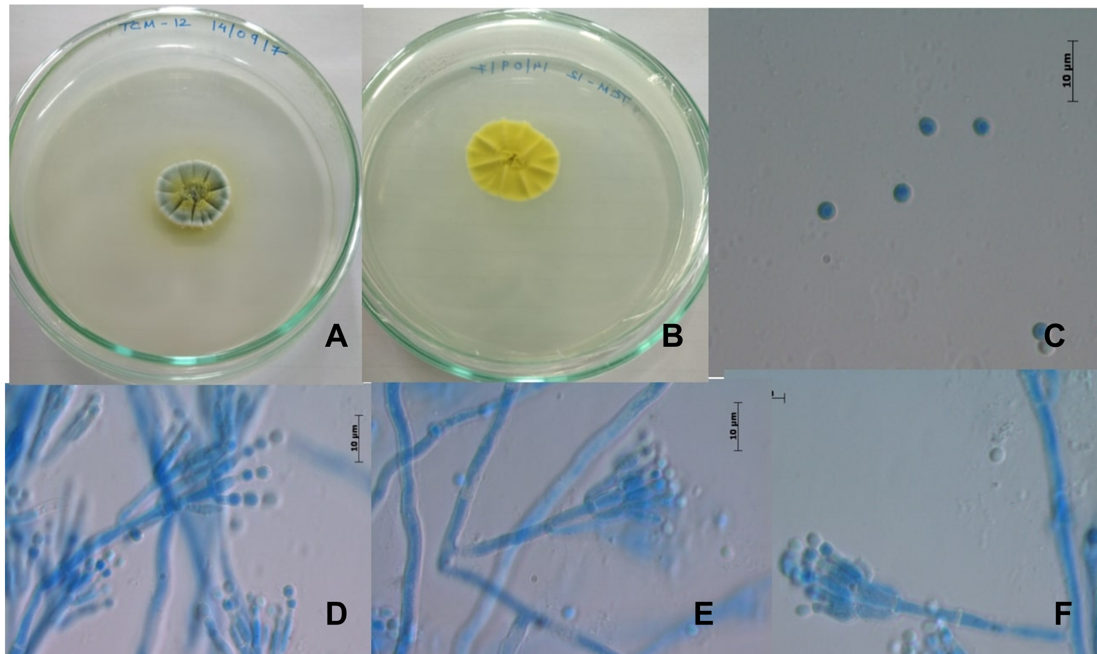

Figure: 37. *Penicillium chrysogenum* (TCM-12) colony on MEA surface (A) and reverse (B); (C) Spore, (D E, F) mature conidiophores with conidia. Scale bars: A–B = 30 mm, C–F = 10 F.

Source: *Phut* (Arunachal Pradesh)

Isolate code: APM-1 and APM-15

Aspergillus sydowii Thom & Church, The Aspergilli: 147 (1926)

Macroscopic characteristics: Colonies grown on MEA plates produce blue-green colonies with yellowish shades. Texture is woolly, velutinous (soft, dense, velvety surface) (Fig. 38).

Microscopic characteristics: Conidiophores are hyaline, long, and smooth-walled stipes. The vesicles (7.0µm-17µm wide) are clavate (club shaped). Conidiogenous structures are biserate with metulae (2µm-3.5µm) and phialides (2µm–5µm) in size.

Conidial structures resemble penicillate (like Penicillium) heads. Conidia are spherical (2.5µm to 3.5µm in diameter). Presence of hülle cells which are covering cleisthothecia.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: form..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type...........................Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: with inflated apical cells bearing

numerous phialides...*Aspergillus*

Key to species:

1) Conidial heads biseriate, colonies "Deflet blue green"..

....***A. sydowii***

Accession number: MK396473 (*Aspergillus sydowii* APM-1) and **MK396474** (*Aspergillus sydowii* APM-15).

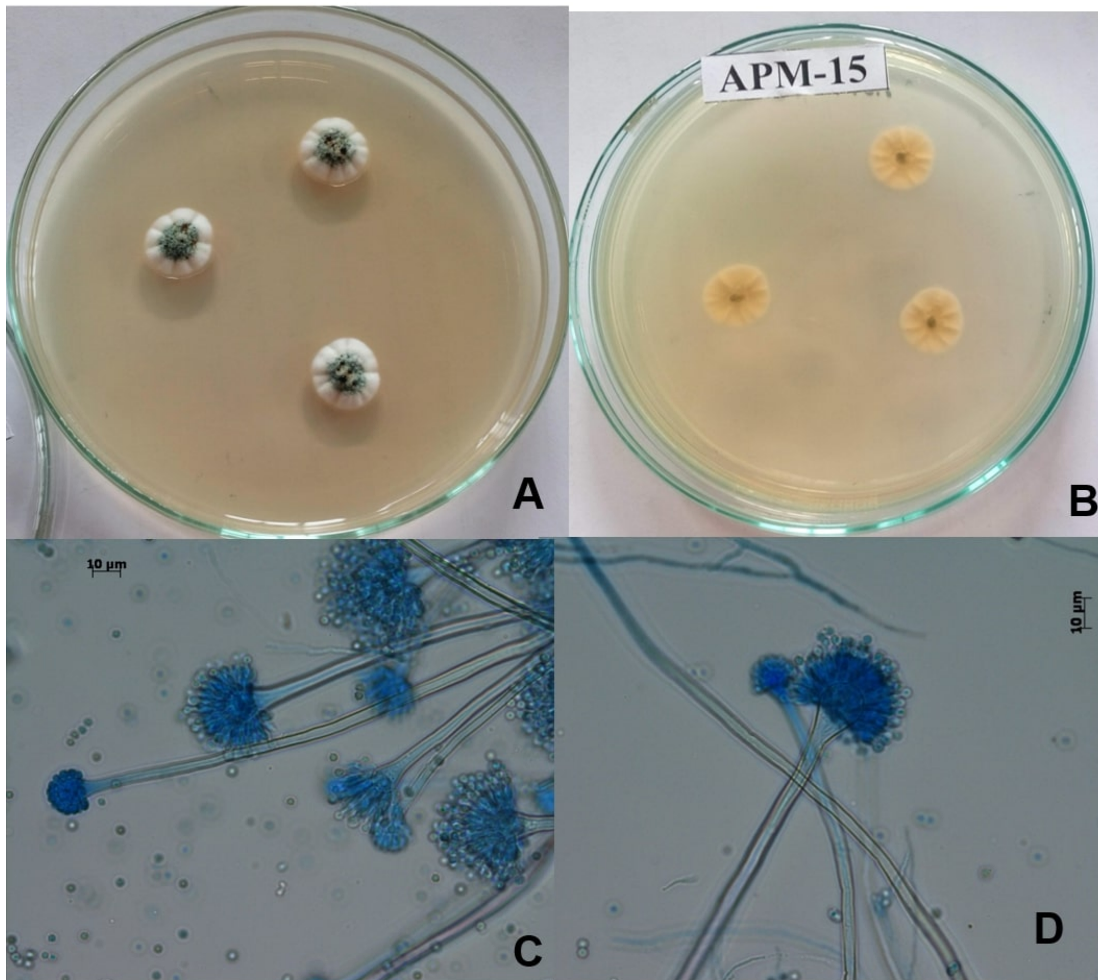

Figure: 38. ***Aspergillus sydowii*** (APM-15) colony on MEA surface (A) and reverse (B); (C, D,) immature conidiophore with vesicle bearing conidiogenous metulae and phialides (biserate). Scale bars: A–B = 30 mm, C=100µM, D–F = 10 µm.

Source: *Phut* (Arunachal Pradesh)

Isolate code: APM-3

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics*:* Colonies on MEA at 28ºC on the fast rise, floccose, light yellow to greyish up to 15 mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 39).

Microscopic characteristics: Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted...*Mucor*

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose..***M. circinelloides***

Accession number: MK396482 (*Mucor circinelloides* APM-3)

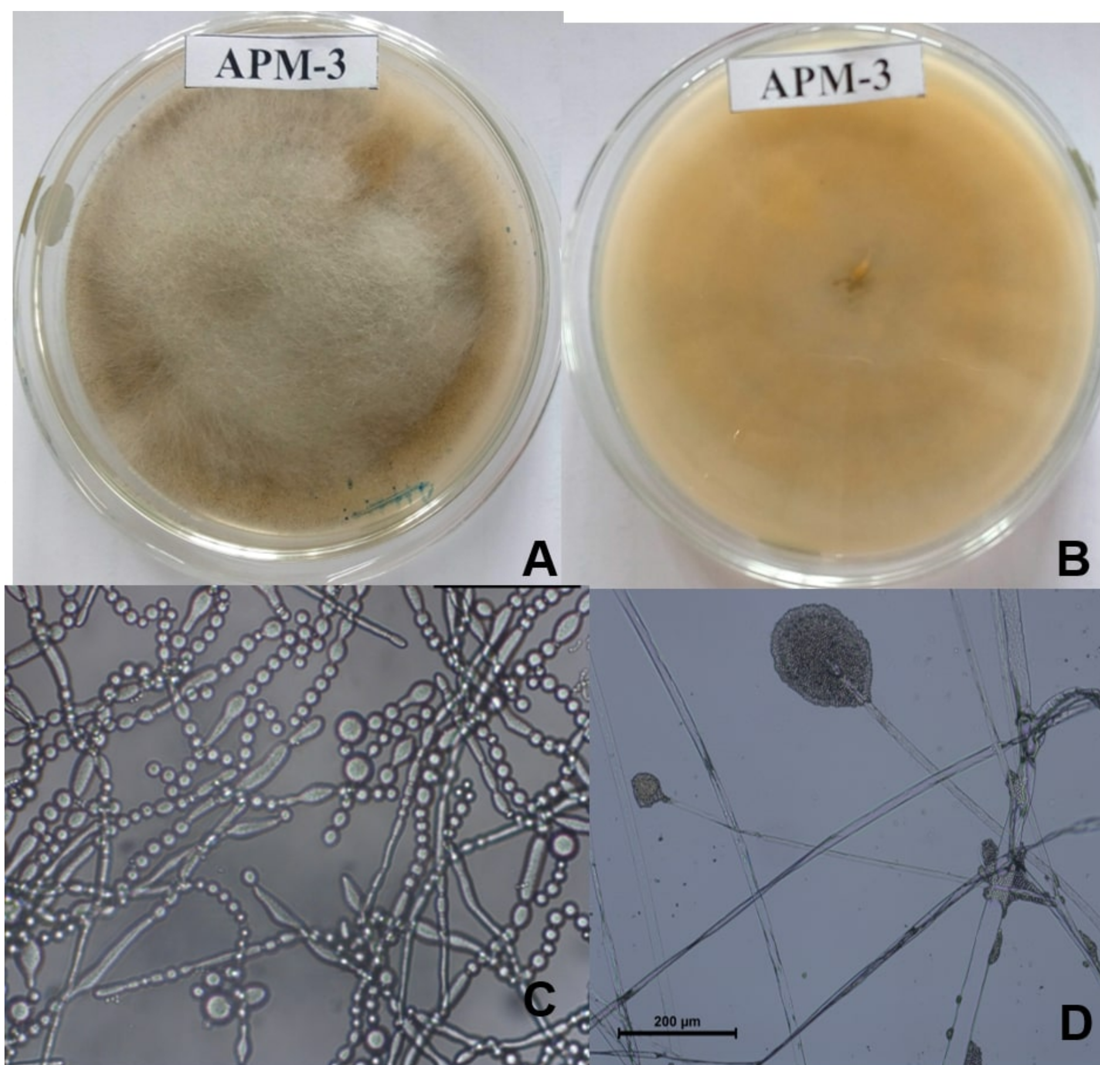

Figure: 39. ***Mucor circinelloides*** (APM-3) colony on MEA surface (A) and (B) spores; (C) Chlamydospore (D) mature sporangiospore. Scale bars: A = 30 mm, C-D = 100 µm.

Source: *Phut* (Arunachal Pradesh)

Isolate code: APM-6

Aspergillus versicolor (Vuill.) Tirab, Annali Bot.: 9 (1908).

Macroscopic characteristics: Colonies on MEA weregreenish-beige to grey green with shades of green to dark green. Exudates present pint to reddish-brown (Fig. 40).

Microscopic characteristics: Smooth conidiophores (200–500µm X 4-7µm) extend from septate hyphae. Conidial heads support vesicles (9-16 µm diameter) which are

biseriate with metulae about the same size, the phialides. The conidiogenous cells (metulae and phialides) loosely cover half of the entire vesicle. Conidia (2.0–3.5µm diameter) are globose (round) and the walls usually have a slightly roughened appearance. Reverse colony is pale to brown in colour.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed

3) Conidia: phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: with inflated apical cells bearing numerous phialides......................

....*Aspergillus*

Key to species:

1) Conidial variably coloured, conidial heads biseriate, sometimes *hulle* cell present

....***Aspergillus versicolour***

Accession number: MK396480 (*Aspergillus versicolour* APM-6)

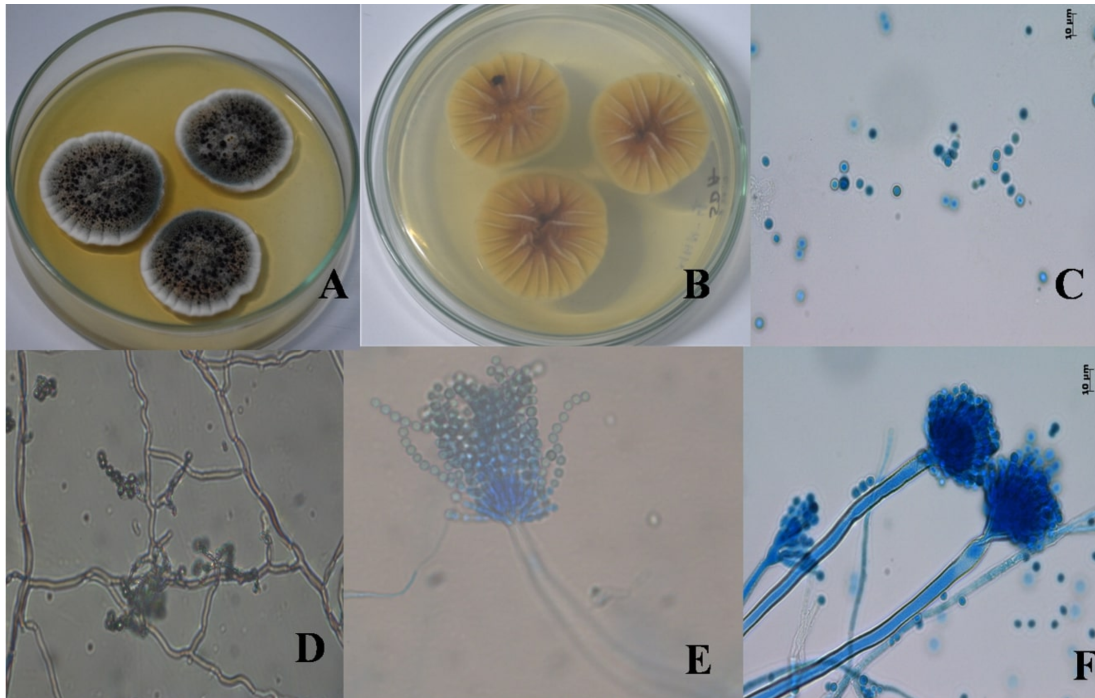

Figure: 40. ***Aspergillus versicolor*** (APM-6) colony on MEA surface (A) and reverse (B); (C) smooth conidiophores; (D) conidial heads supported vesicles with which are biseriate with metulae about the same size of phialides; (E, F) conidiogenous cells (metulae & phialides) loosely cover half to the entire vesicle. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Phut* (Arunachal Pradesh)

Isolate code: APM-7

Mucor indicus Lendn., Bulletin de la Société Botanique de Genève 21: 258 (1930).

Macroscopic characteristics: Colonies on MEA at 28^{0}C fast growing, cottony to fluffy, white to yellow, becoming dark-grey, with the development of sporangia (Fig. 41).

Microscopic characteristics: Sporangiophores are erect, simple or branched, forming large (60-300µm in diameter), terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae. A conspicuous collarette (remnants of the sporangial wall) is usually visible at the base of the columella after sporangiospore dispersal. Sporangiospores are hyaline, grey or brownish, globose

to ellipsoidal and smooth-walled or finely ornamented. Chlamydospores and zygospores also present, mycelia aseptate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted..*Mucor*

Key to the species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose...................................*M. indicus*

Accession number: MK396498 (*Mucor indicus* APM-7)

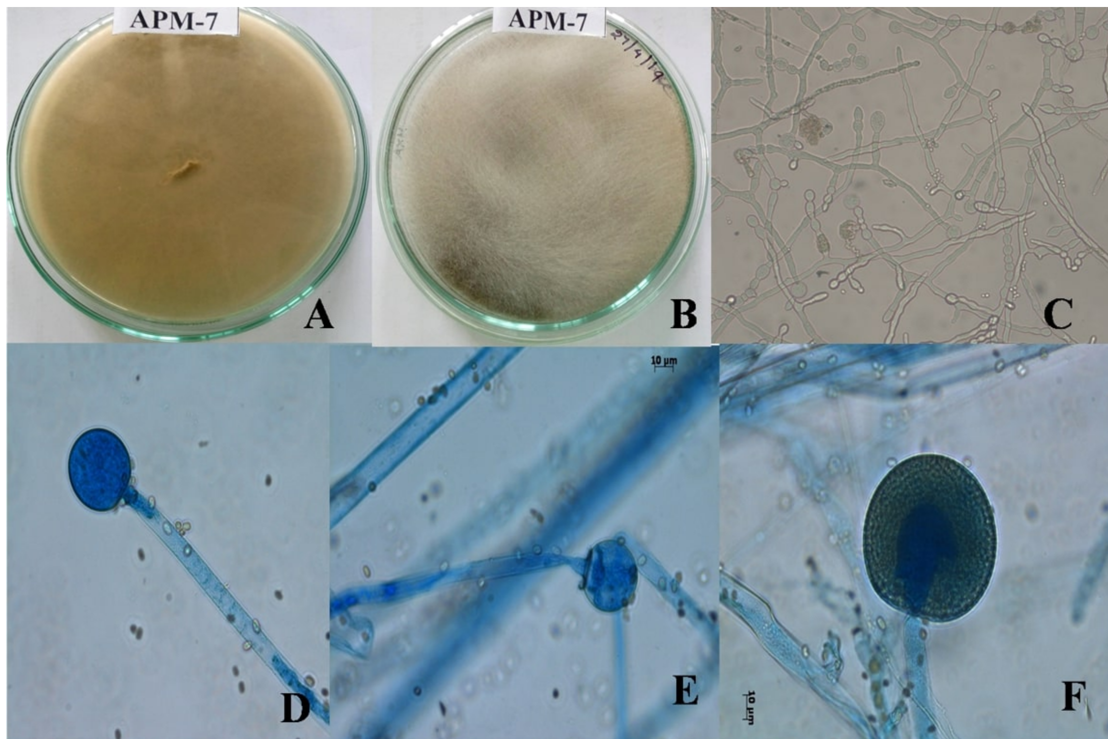

Figure: 41. ***Mucor indicus*** (APM-7) colony pattern on MEA surface (A) and reverse (B); (C) hyphae with chains of chlamydospore (D, E, F) hyphae with apical swelling with mature sporangiospores contain sporangiospores. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Phut* (Arunachal Pradesh)

Isolate code: APM-12

Rhizopus oryzae Went & Prinsen Geerlings., Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 4: 16 (1895).

Macroscopic characteristics: Colonies on MEA at 28^{0}C whitish becoming brownish grey with maturity and reverse side of colony white in colour (Fig. 42).

Microscopic characteristics: Aseptate hyphae with rhizoids brownish, opposite the sporangiospore. Sporangiophore globose and smooth walled becoming dark brown to black brown in colour.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed .... Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not twisted

6) Rhizoid: formed just below

7) Sporangiophore .... *Rhizopus*

Key to species:

1) Sporangiophore striate. Sporangiophore variable in length, up to 4mm long Growth at 37ºC stolon with chlamydospore. Sporangiophores mostly 1-1.5mm long .... ***R. oryzae***

Accession number: MK396490 (*Rhizopus oryzae* APM-12).

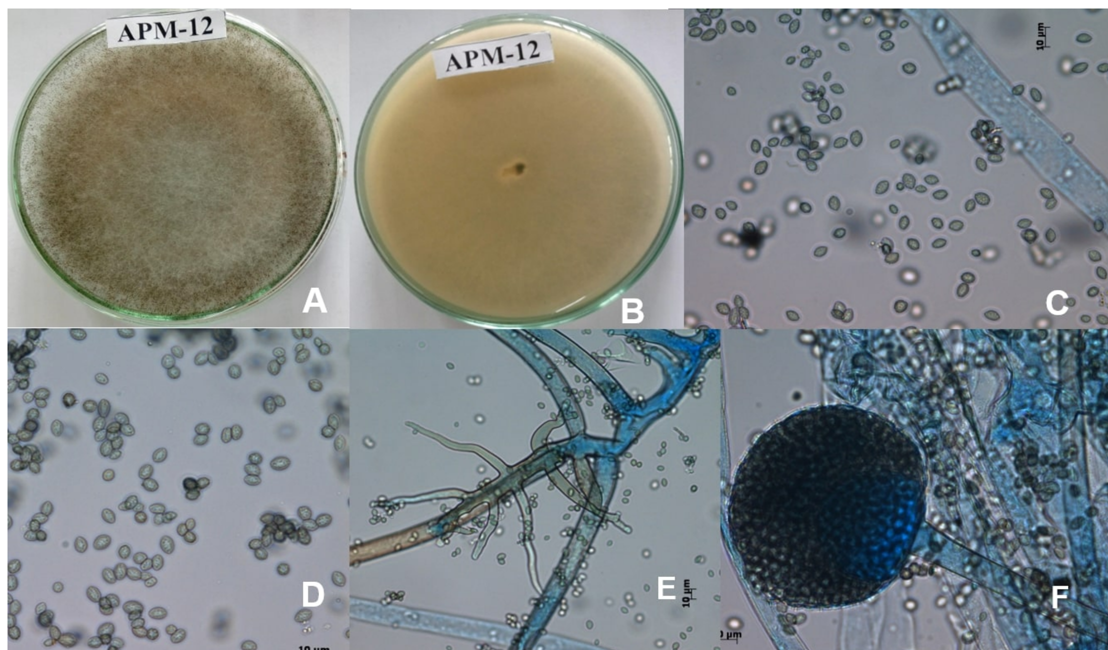

Figure: 42. *Rhizopus oryzae* (APM-12) colony pattern on MEA surface (A) and reverse (B); Spores; (C, D) Branched rhizoids (E). Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Dawdim* (Mizoram)

Isolate code: MDM-1, MDM-14

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics: Colonies on MEA at 28ºC on the rise fast, floccose, light yellow to greyish up to 15 mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 43).

Microscopic characteristics: Aseptate hyphae with sporangiospores hyaline, ellipsoidal and branched. Columella ovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed...Zygomycetes

Key to zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted...*Mucor*

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose............................ ***M. circinelloides***

Accession number: MK396497 *Mucor circinelloides* (MDM-1), **MK396486** (*Mucor circinelloides* (MDM-14).

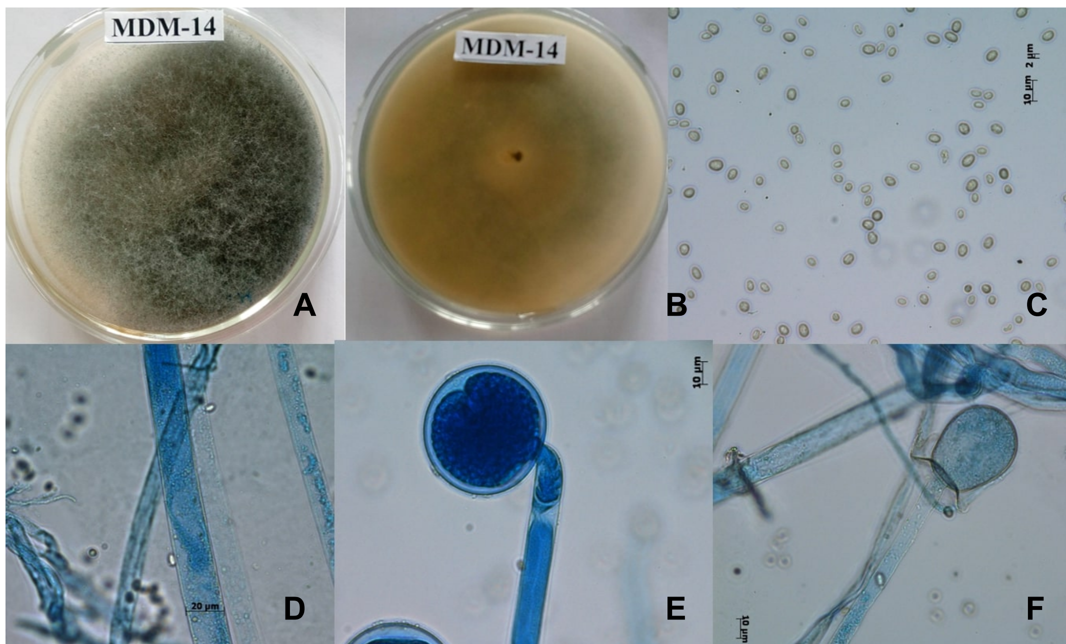

Figure: 43. ***Mucor circinelloides*** (MDM-14) colony on MEA surface (A); reverse (B), (C) Spore, (D) Hyphae (E, F) mature globose sporangiospore. Scale bars: A–B = 30 mm, C= 50µm, D–F = 10 µm.

Source: *Dawdim* (Mizoram)

Isolate code: MDM-10, MDM-16

Bjerkandera adusta P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 5: 38 (1879).

Macroscopic characteristics: Colonies grown on MEA at 28 C for 6-7 days show maximum growth around 8.0 mm in diameter, aerial mycelium abundant, woolly, initially white, later turn to yellowish, reverse white, or yellow (Fig. 44).

Microscopic characteristics: Hyphae dichotomously branched, 3-4.5µm diameter and air-borne hyphae dividing into one-celled arthroconidia which remain cylindrical or become ellipsoidal or slightly barrel-shaped.

Key to class:

1) Hyphae: septate with clamp connection .... Basidiomycetes

Key to species:

1) Pores 6–7 per mm, pore surface white (tube mouths) and smoky-gray to grayish-black tinges, dense zone above tubes ....

.... ***B. adusta***

Accession number: MK396493 (*Bjerkandera adusta* MDM-10), **MK396499** (*Bjerkandera adusta* MDM-16).

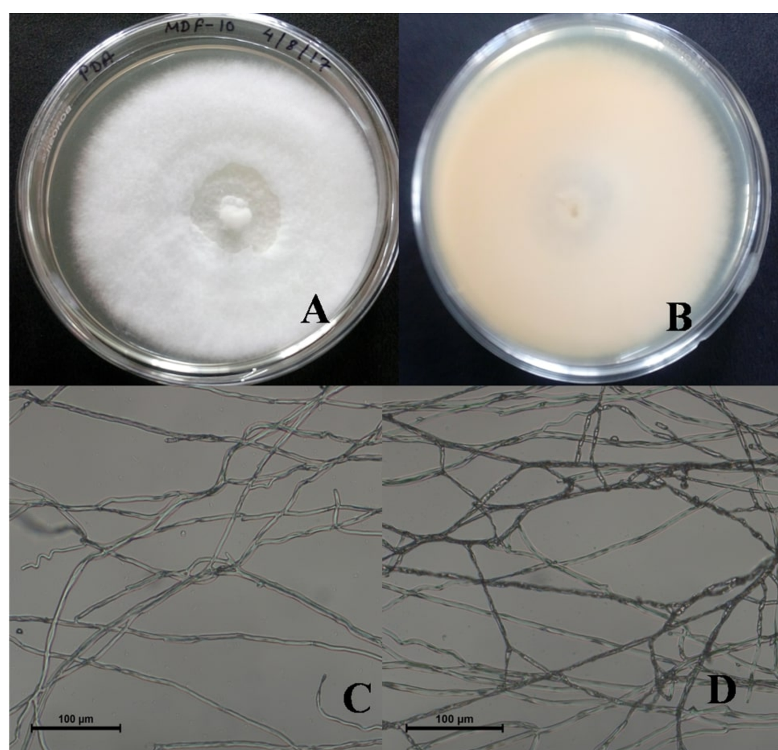

Figure: 44. *Bjerkandera adusta* (MDM-10) colony pattern on MEA surface (A) and reverse (B); (C, D) hyphal structure dichotomously branched.

Source: *Dawdim* (Mizoram)

Isolate code: MDM-11

Rhizopus microsporus Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 83 (1875).

Macroscopic characteristics: Initially colour of colony on MEA is white, its colour slowly changes grey to blackish after 2-3 days at 28ºC (Fig. 45).

Microscopic characteristics: Stolon well developed subhyaline to light brown or grayish brown, aseptate, swollen at the point where rhizoids are formed. Rhizoids are branched and variable in length. Sporangiophore arising from stolon and directly from aerial hyphae, solitary or 2-3 in groups, straight to slightly curved. Sporangia globose, yellowish dark brown when mature. Sporangiophores arising from stolon and opposite to rhizoids, or directly from hyphae. Sporangia globose to depressed globose (20-100µm diameter). Sporangiospores vary or uniform in size and shape, ovoid to sub-globose.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed..Zygomycetes

Key to Zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not twisted

6) Rhizoid: formed just below Sporangiophore..*Rhizopus*

Key to species:

1) Sporangiophores arising directly from hyphae. Sporangia globose (20-100µm diameter). Sporangiospores vary or uniform in size and shape, ovoid to sub-globose ....***R. microsporus***

Accession number: MK396488 (*Rhizopus microsporus* MDM-11).

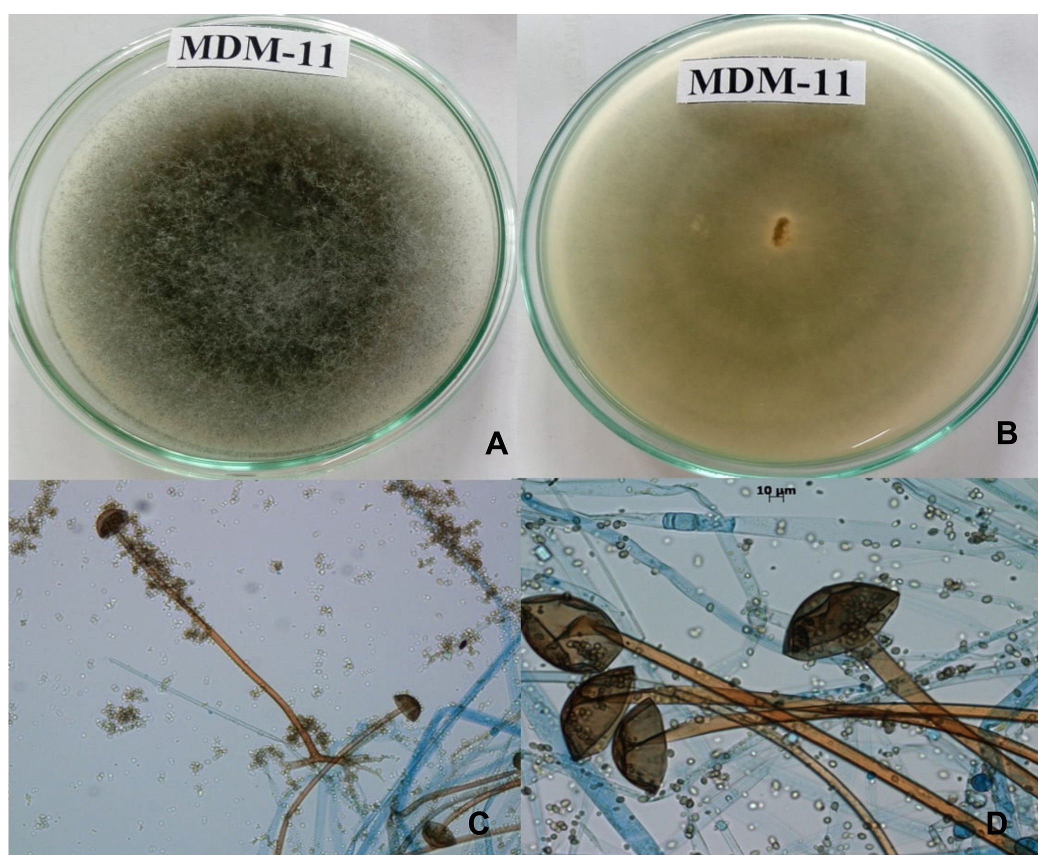

Figure: 45. ***Rhizopus microspores*** (MDM-11) colony pattern on MEA surface (A) and reverse (B); (C, D) well-developed stolons, subhyaline light brown aseptate hyphae and sporangiophore with sporangia and rhizoids formed at swollen point; Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Dawdim* (Mizoram)

Isolate code: MDM-18

Penicillium chrysogenum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 58 (1910).

Macroscopic characteristics: Colonies on MEA grown at 28ºC show a diameter of 3-6 cm within 8 days. Colonies are velvety to floccose with yellow green shade. Exudates typically produce as yellow drops (Fig. 46).

Microscopic characteristics: Conidiophores arising from the substrate, mononematous, usually terverticillate, stipes and smooth walled bearing 4-6 phialides. Phialides flask shaped, often with thick walled. Conidia globose, smooth walled.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type..............................Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: without inflated apical cells

3) Conidia: hyaline

4) Conidia: not so globose

5) Conidia: not so boat shaped

6) Conidia: not so clavate

7) Conidia: dry, not cylindrical

8) Conidiophores: well developed

9) Conidiophores: hyaline, spore aggregate in a row

10) Conidiophores: densely penicillate..*Penicillium*

Key to species:

1) Colonies shade green in colour

2) Conidiophores branched

3) Phialides flask shaped conidiophores stipe smooth walled and terverticillate

4) Conidiophores large up to 700-1600µm tall

5) Colonies velvety, often with yellow exudates and reverse conidia globose..........

....***P. chrysogenum***

Accession number: MK778443 (*Penicillium chrysogenum* MDM-18).

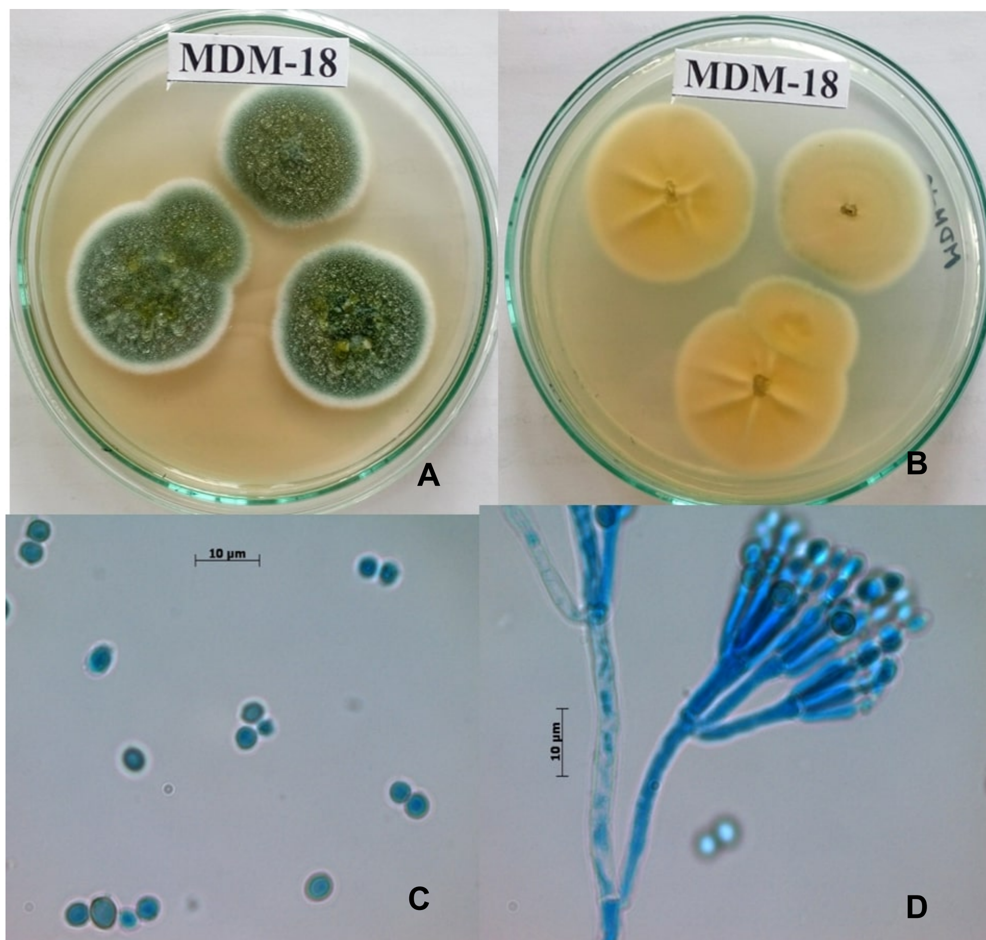

Figure: 46. *Penicillium chrysogenum* (MDM-18) colony on MEA surface (A) and reverse (B); (C) Spore, (D) mature conidiophores with conidia.

Source: *Khekhrii* (Nagaland).

Isolate code: NKM-1

Mucor circinelloides van Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 94 (1875).

Macroscopic characteristics: Colonies on MEA at 28ºC on the fast rise, floccose, light yellow to greyish up to 15 mm height. Sporangiophore turns light yellow, becomes black during maturation stage (Fig. 47).

Microscopic characteristics: Aseptate hyphae with sporangiospores hyaline ellipsoidal and branched. Columella obovoid in large sporangia, globose up to 50µm and few chlamydospore in and on the substrate.

Key to class:

1) Hyphae: aseptate

2) Sporangiospore: formed...Zygomycetes

Key to zygomycetes:

1) Vesicles: not formed

2) Sporangia: globose

3) Sporangia: without apophysis

4) Sporangia: columellate

5) Columella: not so twisted or coiled

6) Rhizoid: not formed

7) Sporangiospore: not partially twisted...*Mucor*

Key to species:

1) Zygospores not formed in single culture not formed

2) Columellae not protuberant

3) Sporangiospores pigmented, sub-globose....................................***M. circinelloides***

Accession number: MK796045 (*Mucor circinelloides* NKM-1).

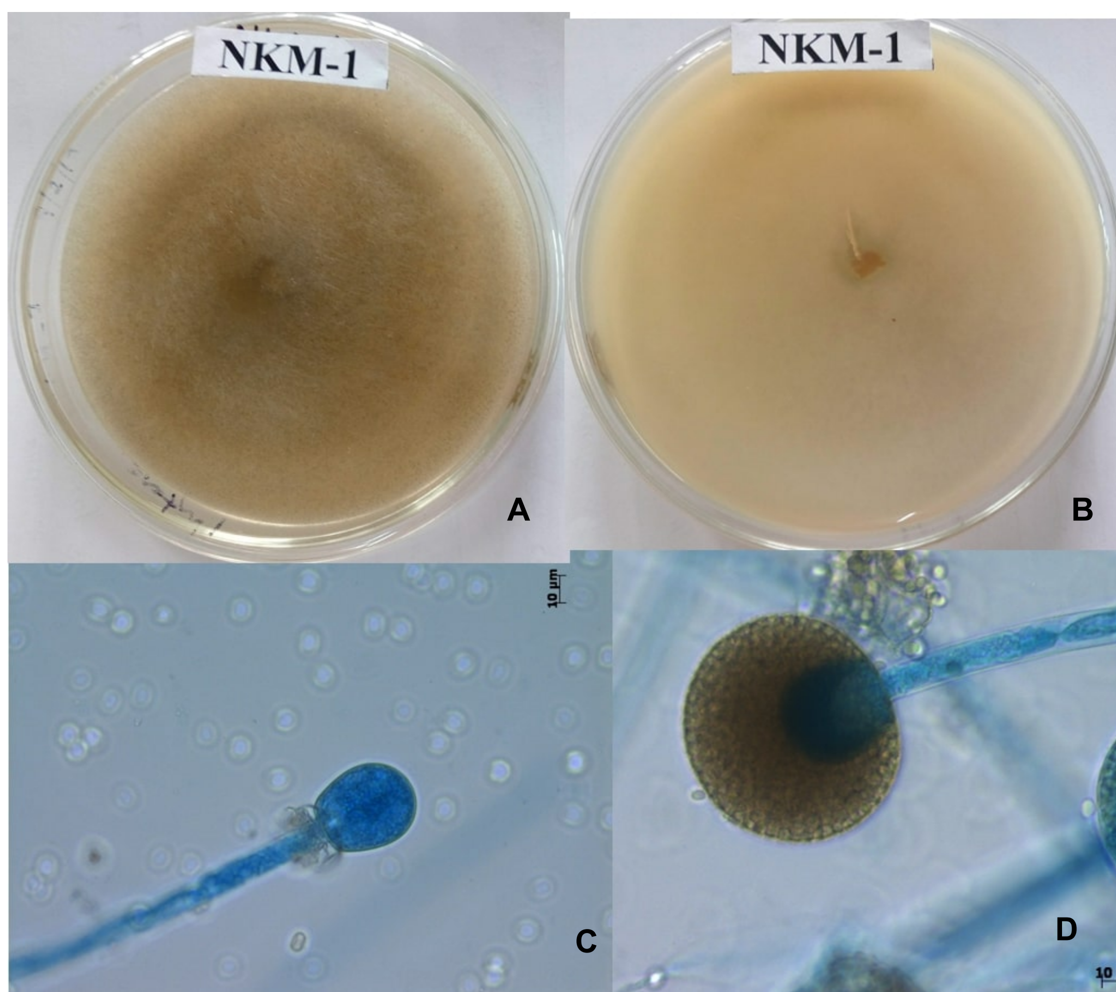

Figure: 47. *Mucor circinelloides* (NKM-1) colony on MEA surface (A); reverse (B), (C) Spore, (D) Hyphae (E, F) mature globose sporangiospore. Scale bars: A–B = 30 mm, C= 50µm, D–F = 10 µm.

Source: *Khekhrii* (Nagaland).

Isolate code: NKM-6

Penicillium citrinum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 61 (1910).

Macroscopic characteristics: Colonies on MEA at 28ºC growing restrictedly, attaining a diameter 1-2 cm within 7-8 days, consisting of a dense felt conidiophore with blue green in colour. Reverse colony change its colour from yellow to orange (Fig. 48).

Microscopic characteristics: Septate hyphae, conidiophores 50-100µm, smooth walled with divergent metulae in a whorl. Metulae bearing 4-5 phialides. Conidia are globose to sub globose, smooth walled and hyaline.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed

3) Conidia: phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidia: hyaline

3) Conidia: not so globose

4) Conidia: not so boat shaped

5) Conidia: not so clavate

6) Conidiophores: well developed

7) Conidia: dry

8) Conidiophores: hyaline, spore aggregate in a row

9) Conidia: not cylindrical

10) Conidia: globose, conidiophores densely penicillate...

....*Penicillium*

Key to species:

1) Colonies shade of green

2) Colonies on CYA growing and sporulating well, conidiophores with distinct long stipe and smaller phialides

3) Conidiophores branched

4) Phialides flask shaped conidiophores stipe smooth walled and biverticillate

5) Colonies restricted, diameter less than 1.7cm within 5-6 days on MEA..***P. citrinum***

Accession number: MK396479 (*Penicillium citrinum* NKM-6).

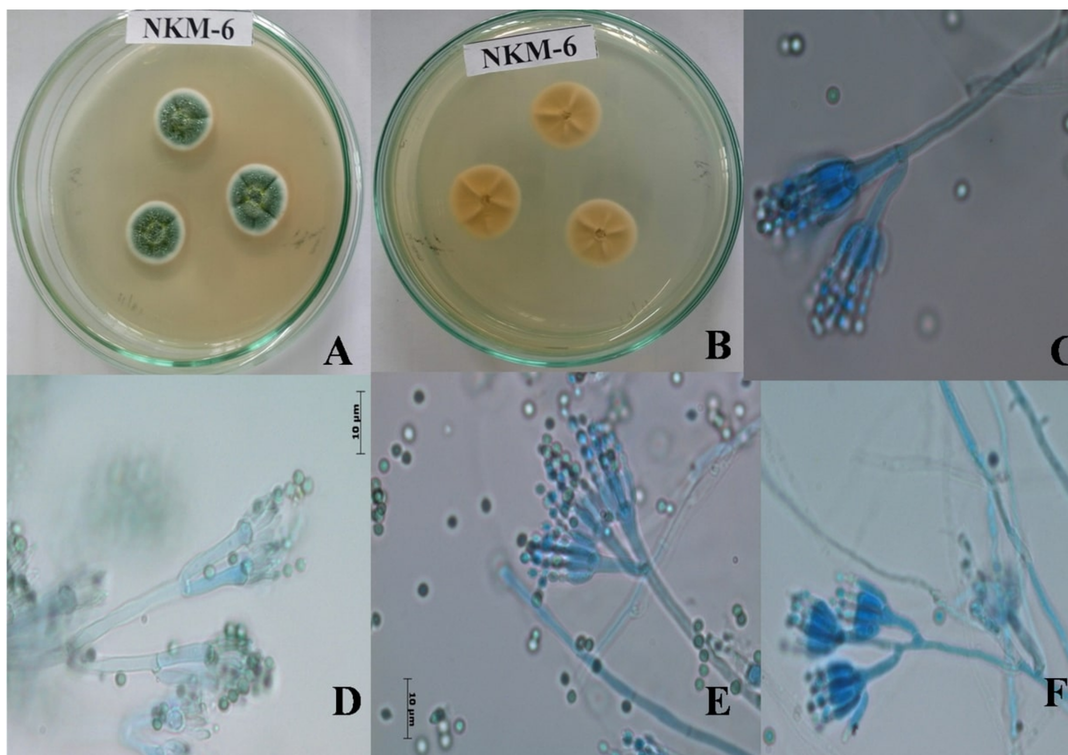

Figure: 48. *Penicillium citrinum* (NKM-6) colony on MEA surface (A) and reverse (B); (C) smooth-walled conidiophores stipes (150–280μm) and biverticillate (D,E,F) phialides ampuliform (flask-shaped) and about 8–12 μm in length, conidia (2.2–3.0 μm diameter) globose to sub-globose. Scale bars: A–B = 30 mm, C–F = 10 μm.

Source: *Khekhrii* (Nagaland)

Isolate code: NKM-7

Aspergillus flavus Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3 (1): 16 1809.

Macroscopic characteristics: Colonies grown on MEA at 28ºC show a diameter of 2-5 cm within 5 days of incubation with olive green colour (Fig. 49).

Microscopic characteristics: Septate hyphae straight, prominent with alternatively, unilaterally or oppositely branched. Appressoria long, near to the distal end of the hyphal cells, alternate or unilateral, single-celled, elongate and attenuate at apex, no haustoria. Conidiophores upright, simple, aseptate and clavate with swelling. Phialides borne directly on the vesicles and radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously coloured in mass, and in dry basipetal chains.

Key to Class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: with inflated apical cells bearing numerous phialides...*Aspergillus*

Key to species:

1) Spore mass: radiate, yellowish green...***A. flavus***

Accession number: MK396470 (*Aspergillus flavus* NKM-7)

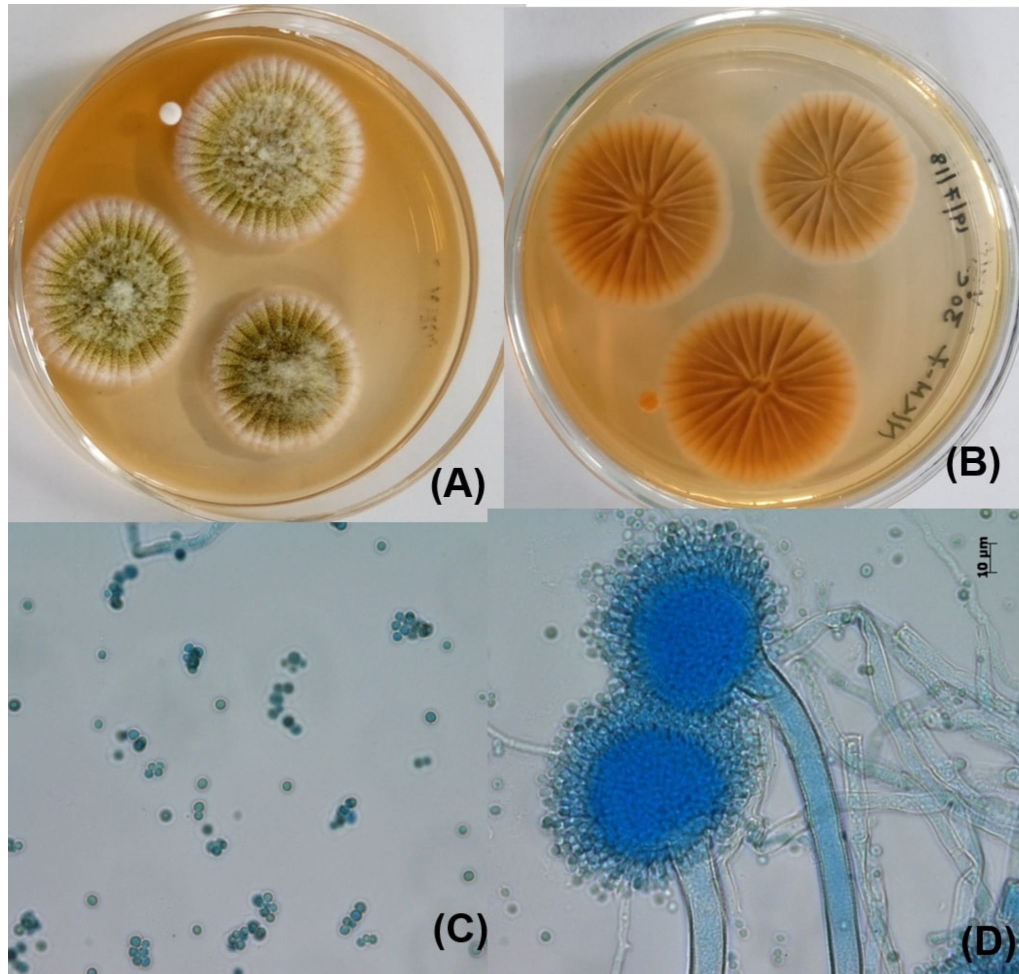

Figure: 49. ***Aspergillus flavus*** (NKM-7) colony on Malt Extract Agar (MEA) surface (A) and reverse (B); (C)septate hyphae with foot cells; (D) conidial head; (E, F) mature globose conidial head contains conidia more typical appearance with phialides radiating from vesicle in all directions. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Khekhrii* (Nagaland)

Isolate code: NKM-8; NKM-13

Aspergillus niger van Tiegh., Annales des Sciences Naturelles Botanique 8: 240 (1867).

Macroscopic characteristics: Colonies on MEA at 28ºC show a diameter of 2-5 cms within 5 days, colonies compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidia are globose to subglobose (3.5-5 µm in diameter), dark brown to black and rough-walled (Fig. 50).

Microscopic characteristics: Septate hyphae straight, prominent with alternatively, unilaterally or oppositely branched. Conidial heads are large (up to 3 mm by 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed and phialospore-type Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: with inflated apical cells bearing numerous phialides....................

....*Aspergillus*

Key to species:

1) Spore mass: black..***A. niger***

Accession number: MK396471 (*Aspergillus niger* NKM-8) and **MK396476** (*Aspergillus niger* NKM-13).

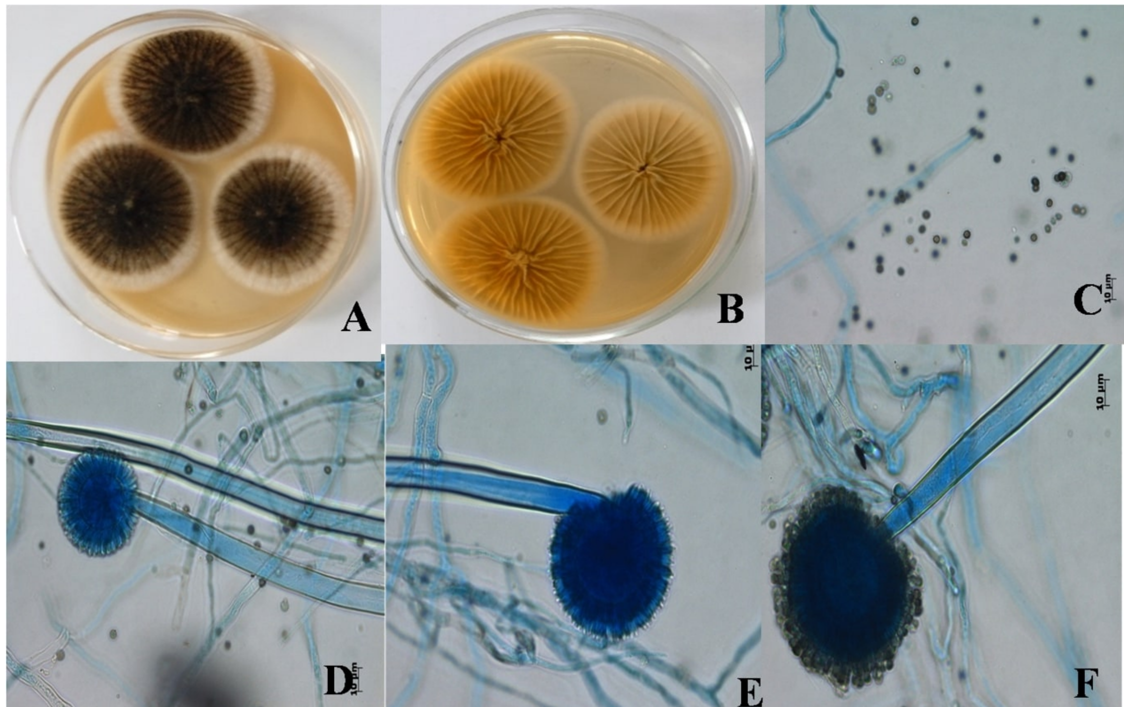

Figure: 50. ***Aspergillus niger*** (NKM-8) colony on MEA surface (A) and reverse (B); (C) conidia; (D, E) large vesicle at end of broken conidiophore bearing metulae and phialides with black pigmented conidia already dispersed; (F) mature conidia globose conidial head contain conidia. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Khekhrii* (Nagaland)

Isolate code: NKM-10

Penicillium oxalicum Currie & Thom, Journal of Biological Chemistry 22: 289 (1915).

Macroscopic characteristics: Colonies on MEA at 28ºC growing attaining a diameter 3-5 cms in 6 days, producing dark green conidia with velutinous colony surface with white edge. Also produce clear exudate droplets with shiny appearance (Fig. 51).

Microscopic characteristics: Septate hyphae, conidiophores arising from the substrate, mononematous, usually biverticilate branched. Stipes, smooth walled, branches, metulae, phialides. Conidia at first sub-globose to ellipsoidal.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: not formed

2) Conidia: formed

3) Conidia: phialospore-type..Phialosporae

Key to Phialosporae:

1) Conidia: 1-celled

2) Conidiophores: without inflated apical cells

3) Conidia: hyaline

4) Conidia: not so globose

5) Conidia: not so boat shaped

6) Conidia: not so clavate

7) Conidiophores: well developed

8) Conidia: dry

9) Conidiophores: hyaline, spore aggregate in a row

10) Conidia: not cylindrical

11) Conidia: globose, conidiophores densely penicillate.......................*Penicillium*

Key to species:

1) Colonies shade green

2) Colonies on CYA growing and sporulating well, conidiophores with distinct long stipe and smaller phialides

3) Conidiophores branched

4) Colonies restricted, diameter less than 1.7cm within 5-6 days on MEA

5) Colonies velvety, conidia large, ellipsoidal 4-6µm long ....

....***P. oxalicum***

Accession number: MK778444 (*Penicillium oxalicum* NKM-10).

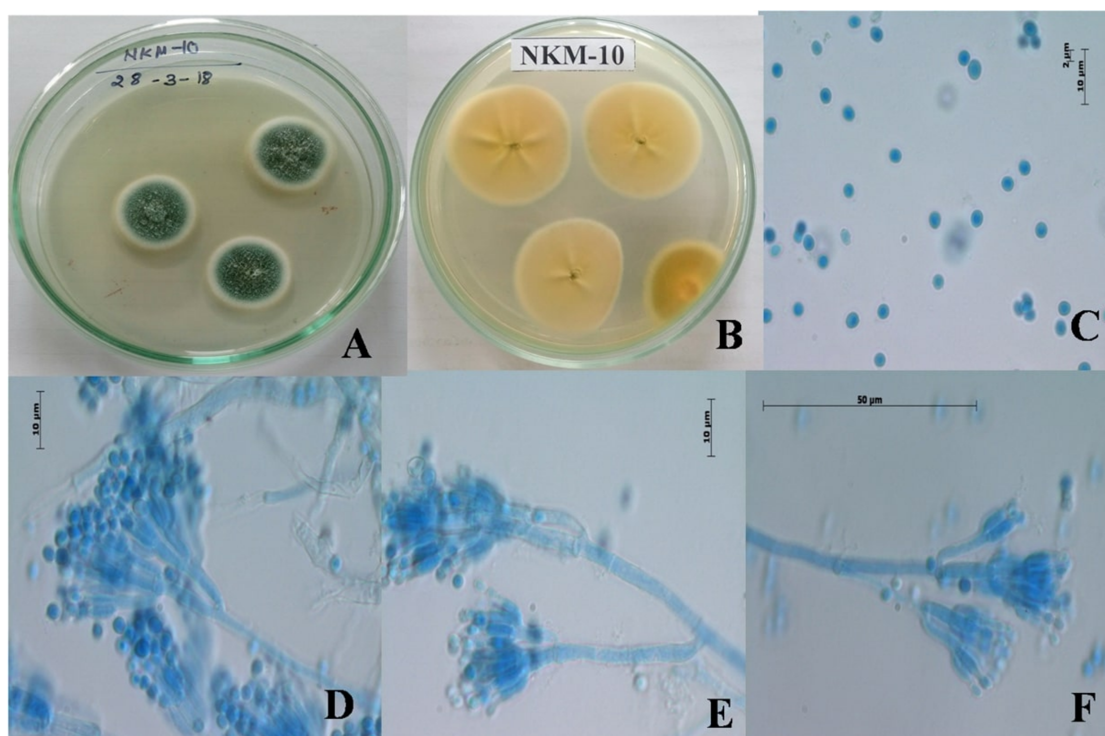

Figure: 51. ***Penicillium oxalicum*** (NKM-10) colony on MEA surface (A) and reverse (B); (C) globose conidia; (D) septate hyphae with penicilli structure; (E, F) mature conidiophores monoverticillate, or biverticillate and asymmetrical, phialides were cylindrical. Scale bars: A–B = 30 mm, C–F = 10 µm.

Source: *Khekhrii* (Nagaland)

Isolate code: NKM-15

Cladosporium parahalotolerans Bensch & Samson, Studies in Mycology 89: 261 (2018).

Macroscopic characteristics: Colonies on MEA show a diameter of 18–40 mm after 14 days at 25°C. Colonies are flat, radially furrowed with crater-like structure, velvety, powdery to felt-woolly, margins white, aerial mycelium diffuse without prominent exudates but sporulation profuse on all media. Colony surface is olivaceous grey and reverse side is olivaceous black-green (Fig. 52).

Microscopic characteristics: Mycelium internal and superficial, hyphae sparingly branched, filiform or narrowly cylindrical-oblong, 1−4µm wide, septate, subhyaline or pale olivaceous brown, almost smooth. Conidiophores macro-, semimacro- and micronematous, arising terminally or laterally from hyphae, filiform or narrowly cylindrical branched, 4−110 × 2−3.5µm, 1−7-septate, septa often darkened where ramoconidia secede, but not constricted, subhyaline, pale olivaceous up to pale medium olivaceous brown.

Key to class:

1) Hyphae: septate without clamp connection

2) Spore: formed

3) Conidia: formed..Deuteromycetes

Key to Deuteromycetes:

1) Conidiomata: formed

2) Conidia: formed

3) Conidia: blastosporae

Key to Blastosporae:

1) Conidiophores: well developed

2) Conidiogenous cells: undifferentiated

3) Conidia: one celled, pigmented, ellipsoidal

4) Conidiophores branched...*Cladosporium*

Key to species:

1) Olivaceous brown in MEA. Conidiophores semimacro- arising terminally filiform or narrowly cylindrical branched, 1−7-septate, septa often darkened where ramoconidia ....***C. parahalotolerans***

Accession number: MK796044 (*Cladosporium parahalotolerans* NKM-15).

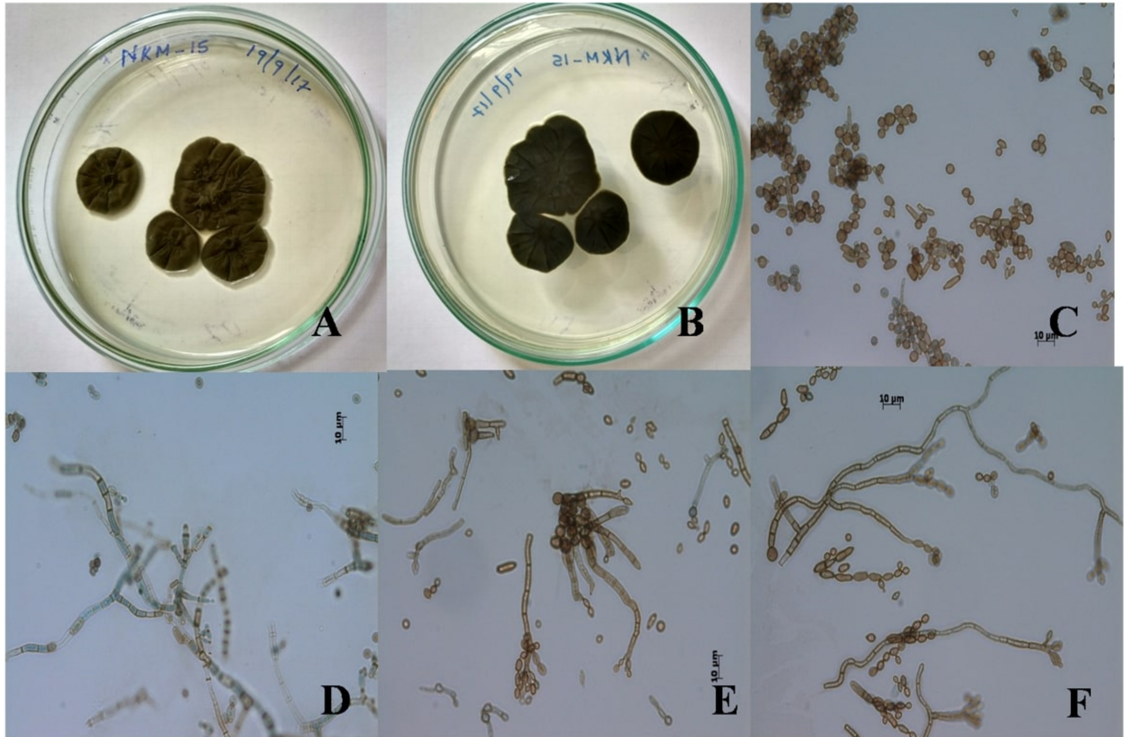

Figure: 52. ***Cladosporium parahalotolerans*** (NKM-15) colony on MEA surface (A) and reverse (B); (C)conidia; (D, E) conidiophores and conidial chain; (F) ramoconidium and conidial chains. Scale bars: A–B = 30 mm, C–F = 10 µm.

Diversity indexes

Diversity indexes of filamentous moulds of dry starters were characterized by species richness (R), Shannon's diversity index (H), and species evenness (E) (Table 12). In the filamentous moulds isolated from *marcha* of Sikkim had the Shannon diversity index (H)-1.74, Species Evenness (E)- 0.97 and Species Richness (R) was calculated as (6). However, *thiat* of Meghalaya had the Shannon diversity index (H) 1.6, Species Evenness (E)- 1, and Species Richness (R) was calculated as 5. Other starter culture *humao* of Assam had the Shannon diversity index (H) 1.56, Species Evenness (E)- 0.96, and Species Richness (R) was calculated as 5. *Hamei* of Manipur had the Shannon diversity index was (H) 0.69, Species Evenness (E)- 1, and Species Richness (R) was calculated as 2. *Chowan* of Tripura had the Shannon diversity index was (H) 1.6, Species Evenness (E)- 1, and Species Richness (R) was calculated as 5. *Phut* of Arunachal Pradesh had the Shannon diversity index was (H) 1.56, Species Evenness (E)- 0.96, and Species Richness (R) was calculated as 5. *Dawdim* of Mizoram has the Shannon diversity index (H) 1.32, Species Evenness (E)- 0.95, and Species Richness (R) was calculated as 4. *Khekhrii* of Nagaland has the Shannon diversity index was (H) 1.46, Species Evenness (E)- 0.82, and Species Richness (R) was calculated as 6. The Shannon diversity index H was recorded highest in *marcha* of Sikkim (H: 1.74) and lowest in *hamei* of Manipur (H: 0.69). Species evenness and lowest in *hamei* of Manipur (H: 0.69). Species Evenness (E) values were 0.97 in *marcha* followed by *humao* of Assam and *phut* of Arunachal Pradesh. The Species Richness (R) values were recorded highest in *marcha* and *khekhrii* samples (Table 12).

Table 12: Frequency, density and diversity Indices of filamentous moulds observed in dry starters of North East India

Filamentous moulds	*Marcha*		*Thiat*		*Humao*		*Hamei*		*Chowan*		*Phut*		*Dawdim*		*Khekhrii*	
	%															
	Fr	RD	Fr	RD	Fr	RD	Fr	RD	Fr	RD	Fr	RD	Fr	RD	Fr	RD
Aspergillus niger	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0.25
Aspergillus flavus	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0	12.5	0.12
Aspergillus sydowii	0	0	16.6	0.16	33.3	0.33	0	0	20	0.2	33.3	0.33	0	0	12.5	0.12
Aspergillus versicolor	0	0		0	0	0	0	0		0	16.6	0.16	0	0	0	0
Penicillium chrysogenum	16.6	0.16	16.6	0.16	0	0	0	0	20	0.2	0	0	16.6	0.16	0	0
Penicillium citrinum	0	0	0	0	16.6	0.16	50	0.5	0	0	0	0	0	0	12.5	0.12
Penicillium oxalicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5	0.12
Cladosporium parahalotolerans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5	0.12
Penicillium polonicum	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mucor circinelloides	16.6	0.16	16.6	0.16	16.6	0.16	50	0.5	20	0.2	16.6	0.16	33.3	0.33	12.5	0.12
Mucor indicus	0	0		0	16.6	0.16	0	0	0	0	16.6	0.16	0	0	0	0
Rhizopus oryzae	0	0		0	16.6	0.16	0	0	20	0.2	16.6	0.16	0	0	0	0
Rhizopus delemar	0	0	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus microsporus	16.6	0.16667		0	0	0	0	0	0	0	0	0	16.6	0.16	0	0
Trametes hirsuta	0	0	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0
Bjerkandera adusta	16.6	0.16667	16.6	0.16	0	0	0	0	20	0.2	0	0	33.3	0.33	0	0
Diversity Indices																
Species Richness (R)	6		5		5		2		5		5		4		6	
Shannon's Diversity Index (H)	1.74		1.6		1.56		0.69		1.6		1.56		1.32		1.46	
Species Evenness (E)	0.97		1		0.96		1		1		0.96		0.95		0.82	

Fr, Frequency of fungal species; RD, Relative density of fungal species in samples

FUNGAL COMMUNITY BY HIGH-THROUGHPUT SEQUENCING

In the present study we selected two popular starters of North East India randomly for profiling the fungal community structure in *marcha* of Sikkim and *thiat* of Meghalaya by using High-throughput amplicon sequencing method. The raw sequence data of two starters of North East were analysed by QIIME1.8 (Quantitative Insights Into Microbial Ecology) software and quality trimming of ITS gene yielded ~0.29 million quality reads for subsequent data analysis. The taxonomic assignment of sequences with the reference database resulted into 5,015 operational taxonomic units (OUTs). The amplicon sequencing of ITS region was found to be 87.5% ± 17.6% (mean ± SD) indicating that majority of the diversity was captured. The sequencing raw data results showed the higher diversity of filamentous moulds in *thiat* whereas *march*a showed the higher yeasts diversity. Yeats and filamentous mould ITS gene sequencing and taxonomic raw data analysis revealed the predominance of yeast phylum *Ascomycota* (98.6%) in *thiat* detected at >1% abundance, whereas the fungal phylum *Mucoromycota* was only detected with 1.4% at >1% abundance (Fig. 53a). However, in *marcha* only yeast phylum *Ascomycota* (100%) was detected at >1% abundance (Fig. 53a). Filamentous moulds phylum was not detected in *marcha* by HTS. Fungal distributions of fungi (filamentous moulds and yeasts) at the family level in *thiat* were *Dothioraceae* (3.94%), *Mucoraceae* (2.63%), *Trichocomaceae* (15.7%) and unidentified fungi (77.73%) (Fig. 53b). The filamentous mould genera distribution in *thiat* were *Aspergillus* (15.7%), *Aureobasidium* (3.9%), *Mucor* (2.7%) and unidentified genera (77.7%) (Fig. 53c). The genera which were unidentified represented the yeast phylum *Ascomycota* in *thiat*. The sequence reads showed that yeast community dominated the *fungal* composition in *marcha* whereas filamentous moulds dominated the fungal composition in *thiat* (Fig. 54).

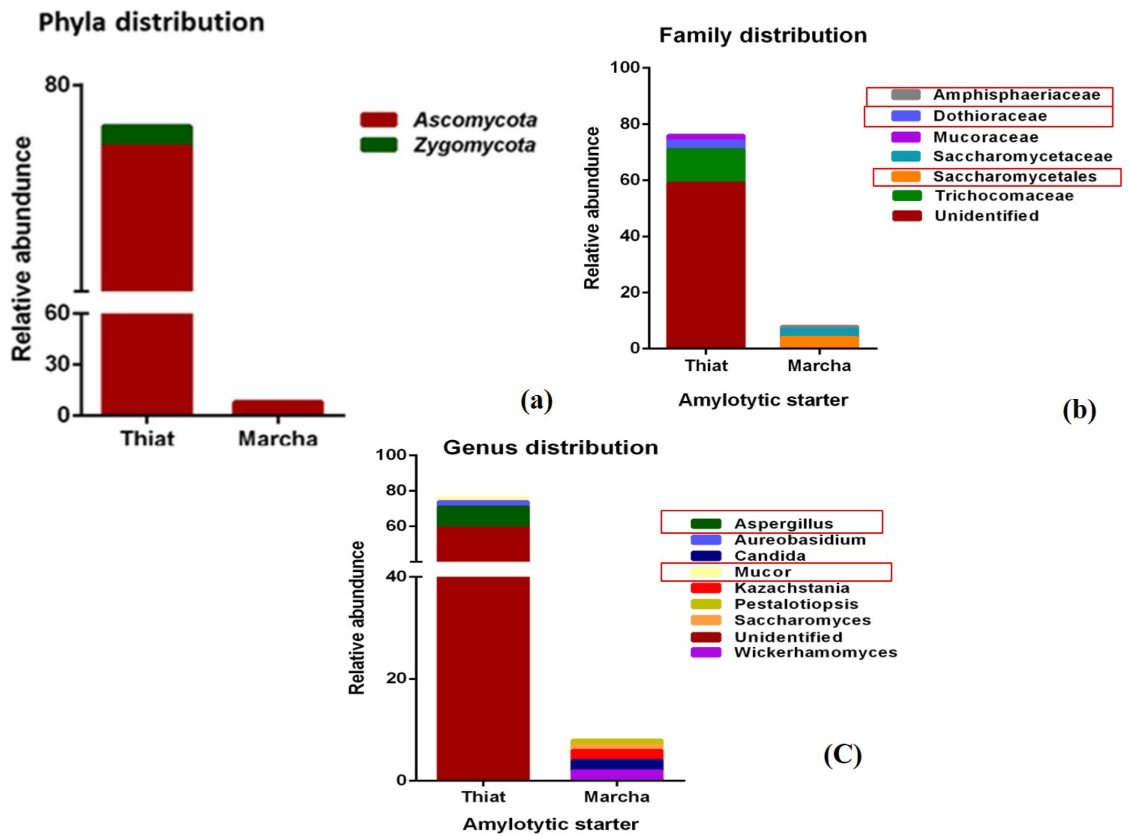

Figure: 53. Filamentous moulds composition in traditional starter cultures *marcha* and *thiat* (a) Phyla; (b) Family and (c) Genus distribution, respectively.

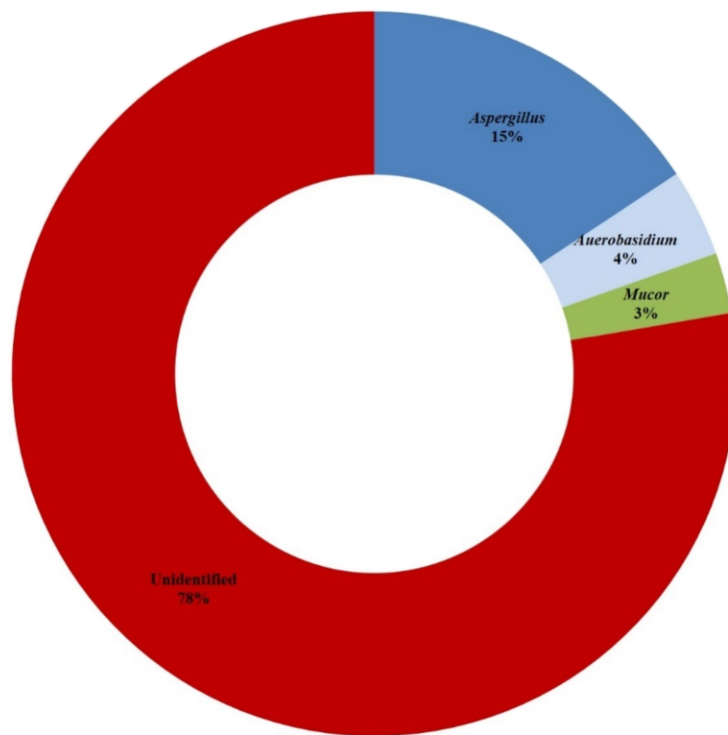

Figure: 54. Filamentous moulds species composition in *thiat*.

Alpha Diversity

Alpha diversity indices revealed significant differences between *thiat* and *marcha* (Table. 13). It was observed that fungal species richness depicted higher in *marcha* (5.25) over *thiat* (5.0). Significant variations were also observed in non-parametric Shannon index for yeast and filamentous mould population follow the reverse trend with *marcha* (2.25) and *thiat* (1.80) (Table 13).

Table: 13. Alpha diversity for *marcha* and *thiat* based on High-throughput Sequence analysis

Starter	Chao1	Goods coverage	Shannon	Simpson
Marcha	5.25	0.75	2.25	0.78125
Thiat	5	1	1.802366931	0.671398892

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ENZYMATIC ACTIVITIES OF FILAMENTOUS MOULDS

Growth at Different Temperatures

For convenience, the filamentous moulds isolated from traditionally prepared dry starters were divided into fast and slow growers, based on the extent of their growth at their optimum temperatures. Fast growers were those showing colony diameter of 30-80 mm within 48-72 h, whereas slow growers took more than 96 h at their optimum temperatures to achieve colony diameters of 10-30 mm (Table 14a-f). *Mucor indicus*, *Mucor circinelloides*, *Rhizopus delemar, Rhizopus microspores* and *Rhizopus oryzae* attained colony diameter between 70-80 mm at their optimum temperatures 72 h (Fig. 55). *Aspergillus flavus, Aspergillus niger, Aspergillus sydowii, Bjerkandera adusta, Penicillium chrysogenum, Penicillium citrinum Penicillium polonicum* and *Trametes hirsuta* attained diameter 12-28 mm at optimum temperature within same time. The slow grower fungus *Cladosporium parahalotolerans* attained colony diameter of 12mm at optimum temperature only after 7 days of incubation at 30ºC. All these filamentous moulds were not able to grow at 5ºC while there were only few filamentous moulds among them which were able to grow at 10ºC such as *Aspergillus flavus, Aspergillus niger, Mucor circinelloides, Mucor indicus, Penicillium chrysogenum, Penicillium citrinum* and *Rhizopus oryzae,* respectively. None of the tested fungi strain grew at 60ºC (Table 14a-f).

Table: 14a. Abilities of fungal strains isolated from dry starters of North East India to grow at different temperature								
Starter	Isolate code	Fungus	Temperature					
			5ºC	10ºC	20ºC	30ºC	40ºC	60ºC
			Colony diameter (mm)					
Marcha	SMM-1	*Aspergillus flavus*	NG	2	2.8	3.2	3.3	NG
	SMM-3	*Mucor circinelloides*	NG	0.8	4.8	FG	NG	NG
	SMM-4	*Rhizopus microsporus*	NG	NG	5.0	FG	FG	NG
	SMM-10	*Bjerkandera adusta*	NG	0.8	2.4	4.6	5.0	NG
	SMM-16	*Penicillium chrysogenum*	NG	0.4	2.1	2.5	NG	NG
	SMM-22	*Penicillium polonicum*	NG	NG	1.2	2.4	NG	NG
	SMM-35	*Penicillium chrysogenum*	NG	0.5	2.2	2.4	NG-	NG

Table: 14b. Abilities of fungal strains isolated from dry starters of North East India to grow at different temperature								
Starter	Isolate code	Fungus	Temperature					
			5ºC	10ºC	20ºC	30ºC	40ºC	60ºC
			Colony diameter (mm)					
Thiat	MTM-1	*Mucor circinelloides*	NG	3.8	FG	FG	1.4	NG
	MTM-4	*Rhizopus delemar*	NG	2.3	FG	FG	FG	NG
	MTM-6	*Penicillium chrysogenum*	NG	0.5	2.4	2.7	NG	NG
	MTM-12	*Trametes hirsuta*	NG	1.7	2.6	3.6	3.9	NG
	MTM-16	*Bjerkandera adusta*	NG	0.6	2.3	4.8	5.3	NG

Table: 14c. Abilities of fungal strains isolated from dry starters of North East India to grow at different temperature

Starter	Isolate code	Fungus	Temperature					
			5ºC	10ºC	20ºC	30ºC	40ºC	60ºC
			Colony diameter (mm)					
Humao	AEM-1	*Penicillium citrinum*	NG	0.9	1.6	2.0	NG	NG
	AEM-3	*Rhizopus oryzae*	NG	3.3	4.2	FG	FG	NG
	AEM-4	*Mucor circinelloides*	NG	1	4.2	FG	FG	NG
	AEM-8	*Aspergillus sydowii*	NG	0.4	1.8	1.9	NG	NG
	AXM-1	*Aspergillus sydowii*	NG	NG	1.3	1.7	3.2	NG
	AMM-3	*Mucor indicus*	NG	3.6	4.8	5.9	FG	NG
Hamei	MHM-1	*Mucor circinelloides*	NG	3.5	FG	FG	FG	NG
	MHM-15	*Penicillium citrinum*	NG	NG	1.8	2.2	NG	NG

Table: 14d. Abilities of fungal strains isolated from dry starters of North East India to grow at different temperature

Starter	Isolate code	Fungus	Temperature					
			5ºC	10ºC	20ºC	30ºC	40ºC	60ºC
			Colony diameter (mm)					
Chowan	TCM-1	*Bjerkandera adusta*	NG	0.3	0.7	3.5	NG	NG
	TCM-4	*Mucor circinelloides*	NG	NG	4.8	FG	FG	NG
	TCM-7	*Rhizopus oryzae*	NG	2.0	FG	FG	FG	NG
	TCM-9	*Aspergillus sydowii*	NG	NG	1.1	1.5	NG	NG
	TCM-12	*Penicillium chrysogenum*	NG	0.4	1.2	2.4	NG	NG
Phut	APM-1	*Aspergillus sydowii*	NG	NG	1.4	1.6	NG	NG
	APM-3	*Mucor circinelloides*	NG	1.3	4.1	FG	FG	NG
	APM-6	*Aspergillus versicolor*	NG	NG	1.9	2.2	2.6	NG
	APM-7	*Mucor indicus*	NG	2.6	4.8	FG	FG	NG
	APM-12	*Rhizopus oryzae*	NG	2.3	FG	FG	FG	NG
	APM-15	*Aspergillus sydowii*	NG	NG	1.1	1.5	NG	NG

Table: 14e. Abilities of fungal strains isolated from dry starters of North East India to grow at different temperature								
Starter	**Isolate code**	**Fungus**	**Temperature**					
			5ºC	**10ºC**	**20ºC**	**30ºC**	**40ºC**	**60ºC**
			Colony diameter (mm)					
Dawdim	MDM-1	*Mucor circinelloides*	NG	1.9	5.2	FG	FG	NG
	MDM-10	*Bjerkandera adusta*	NG	0.2	2.0	3.6	4.9	NG
	MDM-11	*Rhizopus microsporus*	NG	NG	4.8	FG	FG	NG
	MDM-14	*Mucor circinelloides*	NG	1.9	5.1	FG	FG	NG
	MDM-16	*Bjerkandera adusta*	NG	0.5	1.8	2.8	NG	NG
	MDM-18	*Penicillium chrysogenum*	NG	0.6	2.2	2.3	NG	NG

Table: 14f. Abilities of fungal strains isolated from dry starters of North East India to grow at different temperature								
Starter	**Isolate code**	**Fungus**	**Temperature**					
			5ºC	**10ºC**	**20ºC**	**30ºC**	**40ºC**	**60ºC**
			Colony diameter (mm)					
Khekhrii	NKM-1	*Mucor circinelloides*	NG	FG	FG	FG	FG	NG
	NKM-6	*Penicillium citrinum*	NG	1.3	1.8	2.0	NG	NG
	NKM-7	*Aspergillus flavus*	NG	1.8	1.9	2.0	3.2	NG
	NKM-8	*Aspergillus niger*	NG	2.2	3.8	4.3	5	NG
	NKM-10	*Penicillium oxalicum*	NG	NG	1.4	1.5	NG	NG
	NKM-13	*Aspergillus niger*	NG	NG	1.2	1.6	NG	NG
	NKM-15	*Cladosporium parahalotolerans*	NG	NG	0.6	1.2	NG	NG
NG, no growth; FG, full growth								

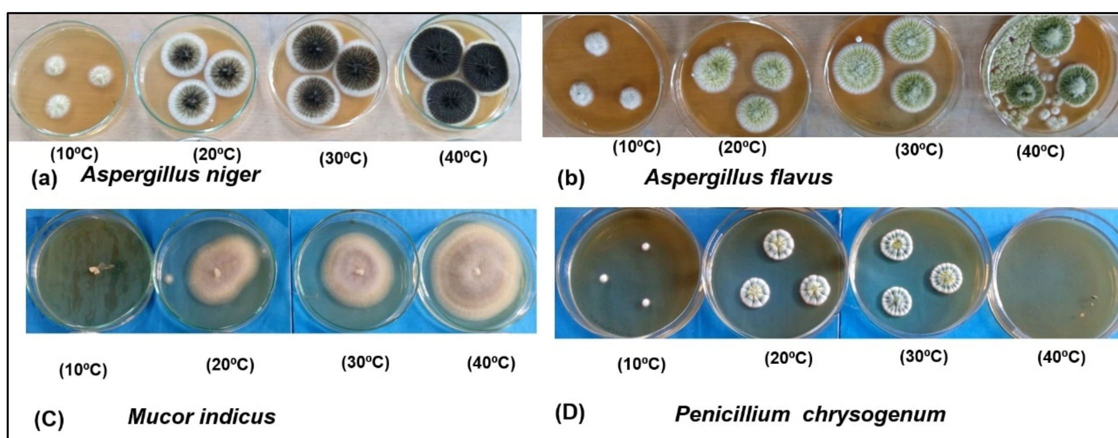


Figure: 55. Mycelial growth of filamentous moulds MEA at different temperatures.

Screening of Extracellular Amylase Activity

Extracellular enzymatic characteristics that are believed to be important for traditional fermentation, such as amylase, cellulase, L-asparaginase, lipase, protease and xylanase activities (Raveendran et al. 2018; Alam et al. 2019 Kumar et al. 2019), were analysed for filamentous moulds. During qualitative screening of extracellular amylase producers, all 44 filamentous moulds isolated from dry starters of North East India were evaluated for their potential to produce and secrete amylase enzyme (Table 15). Out of 44 strains of filamentous moulds, 22 fungal strains showed amylolytic activities on starch agar by observing clear zone of starch hydrolysis (Fig 56a). Among all isolates *Aspergillus niger* (NKM-8) isolated from *khekhrii* and *Rhizopus oryzae* (AEM-3) from *humao* showed maximum zone of inhibition (Table 15). *Aspergillus flavus* and *Aspergillus niger* (NKM-8 and NKM-13) from *khekhrii*, *Aspergillus sydowii* (AEM-8, AXM-1, TCM-9, APM-1 and APM-15) from *humao, chowan* and *phut* and *Aspergillus versicolour* (APM-6) from *phut* were selected for quantitative evaluation of amylase activity. The genus *Penicillium chrysogenum* (SMM-16, SMM-35, TCM-12, MDM-18, MTM-6) from *marcha, chowan, dawdim* and *thiat,* respectively. *Penicillium*

citrinum (AEM-1, MHM-15, and NKM-6) from *humao, hamei* and *khekhrii* and *Penicillium oxalicum* (NKM-10) from *khekhrii* was well screen for amylase enzyme. Filamentous moulds *Rhizopus oryzae* (AEM-3, TCM-7 and APM-12) from *humao, chowan* and *phut* also selected for amylase activity (Table 15; Fig. 56a).

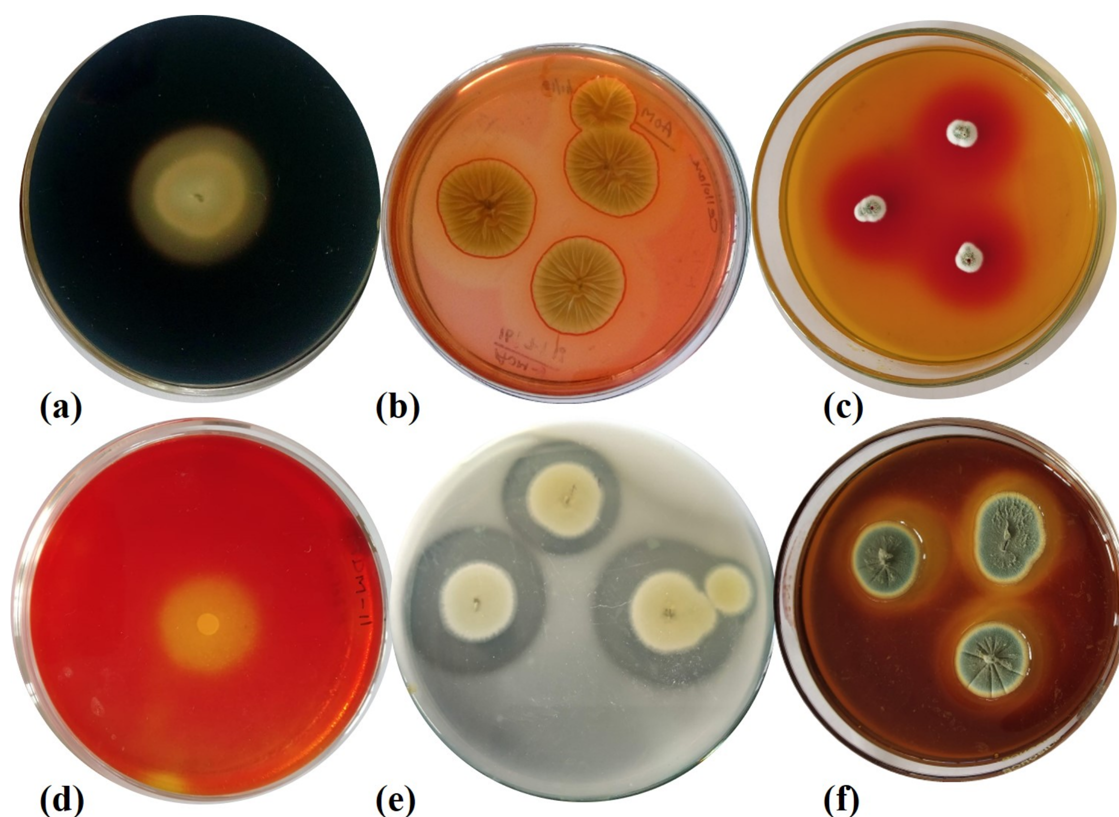


Figure: 56. Plates (A) Display filamentous colonies developing clear zones on 1% starch containing medium. (B) Detection of cellulase activity from the isolated filamentous moulds on agar plates. Plates showing the zone of hydrolysis by filamentous moulds with Congo red staining. (C) Displaying the filamentous moulds developing clear zone tannin containing medium (D) Displaying the filamentous moulds developing clear zone tannin containing medium (E) Detection of protease activity from the filamentous moulds on gelatin agar plate. (F) Enzyme activity screening of fungal isolates; zone of clearance on xylan plate.

Table: 15. Screening of extracellular enzymes by fungal strains isolated from starters of North East India

Starter	Isolate code	Identity	Diameter in mm										
			Amylase		**Cellulase**		**L- Asparaginase**		**Lipase**	**Protease**		**Xylanase**	
			Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance
Marcha	SMM-1	*Aspergillus flavus*	10	30	-	-	15	28	19	27	38	18	39
	SMM-3	*Mucor circinelloides*	-	-	-	-	35	58	-	-	-	-	-
	SMM-4	*Rhizopus microsporus*	-	-	-	-	34	50	24	-	-	-	-
	SMM-10	*Bjerkandera adusta*	-	-	-	-	19	32	-	-	-	18	30
	SMM-16	*Penicillium chrysogenum*	15	22	-	-	13	26	-	15	32	17	41
	SMM-22	*Penicillium polonicum*	-	-	12	24	-	-	-	-	-	-	-
	SMM-35	*Penicillium chrysogenum*	16	23	-	-	14	22	-	16	29	17	43
Thiat	MTM-1	*Mucor circinelloides*	-	-	-	-	37	49	-	-	-	-	-
	MTM-4	*Rhizopus delemar*	-	-	-	-	48	68	-	-	-	-	-
	MTM-6	*Penicillium chrysogenum*	15	22	-	-	10	17	-	17	32	15	42
	MTM-12	*Trametes hirsuta*	-	-	10	30	-	-	-	-	-	17	22
	MTM-16	*Bjerkandera adusta*	-	-	-	-	14	36	-	-	-	15	29

Table: 15. Screening of extracellular enzymes by fungal strains isolated from starters of North East India

Starter	Isolate code	Identity	Diameter in mm										
			Amylase		Cellulase		L- Asparaginase		Lipase	Protease		Xylanase	
			Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance
Humao	AEM-1	*Penicillium citrinum*	13	28	-	-	12	21	21	-	-	21	34
	AEM-3	*Rhizopus oryzae*	30	38	-	-	28	40	38	-	-	-	-
	AEM-4	*Mucor circinelloides*	-	-	-	-	36	56	-	-	-	-	-
	AEM-8	*Aspergillus sydowii*	15	24	15	28	15	29	-	10	29	-	-
	AXM-1	*Aspergillus sydowii*	16	25	16	27	14	27	-	8	25	-	-
	AMM-3	*Mucor indicus*	-	-	-	-	-	-	-	-	-	28	36
Hamei	MHM-1	*Mucor circinelloides*	-	-	-	-	34	46	-	-	-	-	-
	MHM-15	*Penicillium citrinum*	9	22	-	-	11	21	19	-	-	19	43
Chowan	TCM-1	*Bjerkandera adusta*	-	-	-	-	15	29	-	-	-	13	27
	TCM-4	*Mucor circinelloides*	-	-	-	-	25	35	-	-	-	-	-
	TCM-7	*Rhizopus oryzae*	31	39	-	-	22	39	36	-	-	-	-
	TCM-9	*Aspergillus sydowii*	17	26	15	28	12	21	-	12	31	-	-

Table: 15. Screening of extracellular enzymes by fungal strains isolated from starters of North East India

Starter	Isolate code	Identity	Diameter in mm										
			Amylase		**Cellulase**		**L- Asparaginase**		**Lipase**	**Protease**		**Xylanase**	
			Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance
	TCM-12	*Penicillium chrysogenum*	16	29	-	-	16	25	-	12	28	19	43
Phut	APM-1	*Aspergillus sydowii*	12	25	16	26	17	26	-	11	25	-	-
	APM-3	*Mucor circinelloides*	-	-	-	-	39	54	-	-	-	-	-
	APM-6	*Aspergillus versicolor*	15	22	-	-	13	19	-	22	56	-	-
	APM-7	*Mucor indicus*	-	-	-	-	-	-	-	-	-	22	35
	APM-12	*Rhizopus oryzae*	32	39	-	-	20	36	39	-	-	-	-
	APM-15	*Aspergillus sydowii*	14	26	14	24	16	25	-	10	18	-	-
Dawdim	MDM-1	*Mucor circinelloides*	-	-	-	-	23	42	-	-	-	-	-
	MDM-10	*Bjerkandera adusta*	-	-	-	-	12	26	-	-	-	16	21
	MDM-11	*Rhizopus microsporus*	-	-	-	-	29	45	29	-	-	-	-
	MDM-14	*Mucor circinelloides*	-	-	-	-	37	49	-	-	-	-	-

Table: 15. Screening of extracellular enzymes by fungal strains isolated from starters of North East India

Starter	Isolate code	Identity	Diameter in mm										
			Amylase		Cellulase		L- Asparaginase		Lipase	Protease		Xylanase	
			Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance
	MDM-16	*Bjerkandera adusta*	-	-	-	-	13	27	-	-	-	19	32
	MDM-18	*Penicillium chrysogenum*	16	23	-	-	15	23	-	19	34	18	32
Khekhrii	NKM-1	*Mucor circinelloides*	-	-	-	-	34	42	-	-	-	-	-
	NKM-6	*Penicillium citrinum*	10	21	-	-	12	18	18	-	-	24	46
	NKM-7	*Aspergillus flavus*	11	38	-	-	13	25	22	28	51	17	32
	NKM-8	*Aspergillus niger*	14	40	-	-	-	-	-	-	-	-	-
	NKM-10	*Penicillium oxalicum*	15	24	-	-	-	-	-	-	-	-	-
	NKM-13	*Aspergillus niger*	12	42	-	-	-	-	-	-	-	-	-
	NKM-15	*Cladosporium parahalotolerans*	-	-	-	-	8	18	-	-	40	14	29

-, no colony of fungi and no inhibition zone

After primary screening, all 22 filamentous moulds were assessed for their quantitative estimation under submerged fermentation conditions. *Aspergillus niger* (NKM-8) isolated from *khekhrii* showed maximum amylase activity of 27.67 U/ml, followed by *Aspergillus niger* (NKM-13) isolated from *khekhrii* 26.7 U/ml, and *Aspergillus flavus* (SMM-1) isolated from *marcha* 22.06 U/ml, respectively (Fig. 57).

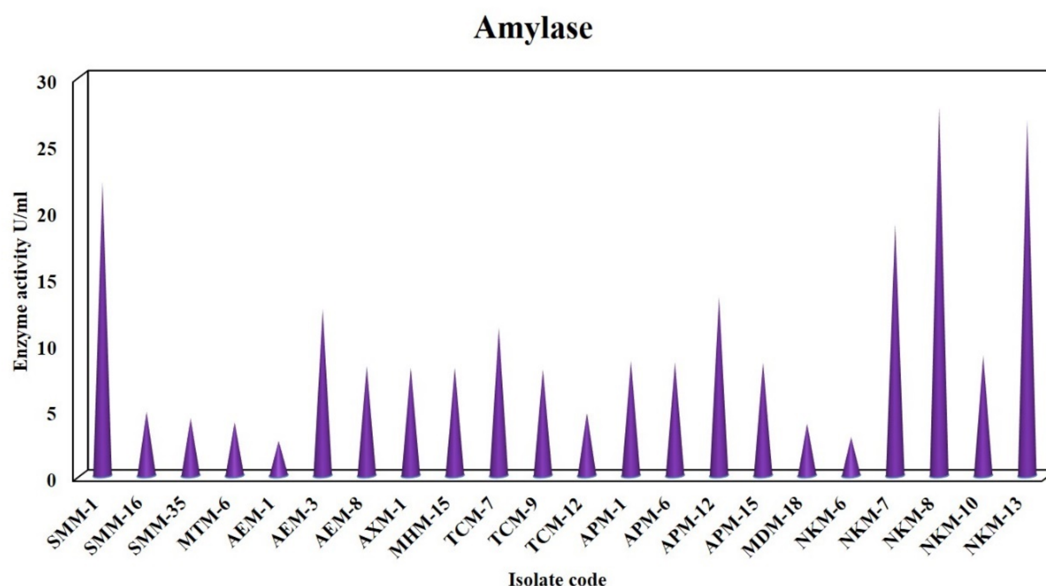


Figure: 57. Screening of filamentous moulds for their ability to produce amylase enzyme.

Screening of Extracellular Cellulase Activity

A total of 44 filamentous moulds were screened for the extracellular cellulase enzyme activity out of which only 7 isolates only showed cellulase activity (Table 15). *Trametes hirsuta* (MTM-12) isolated from *thiat* showed the high cellulase activity; its clear zone was approximately 30 mm in diameter. Also, *Aspergillus sydowii* (AEM-8, AXM-1, TCM-9, APM-1 and APM-15) isolated from *humao, chowan* and *put,* respectively*; Penicillium polonicum* (SMM-22) isolated from *marcha* also showed positive result against cellulase enzyme (Table 15; Fig 56b). The quantitative estimation of cellulase enzyme activity was done by DNS method (Legodi et al. 2019). *Trametes hirsuta* (MTM-12) isolated from *thiat* was found to be most potential filamentous moulds for cellulase enzyme activity (15.6

U/ml) (Fig. 58), followed by *Aspergillus sydowii* isolated from *humao* (AXM-1) 10.8 U/ml and *Penicillium polonicum* isolated from *marcha* (SMM-22) 10.1 U/ml respectively (Fig. 58).

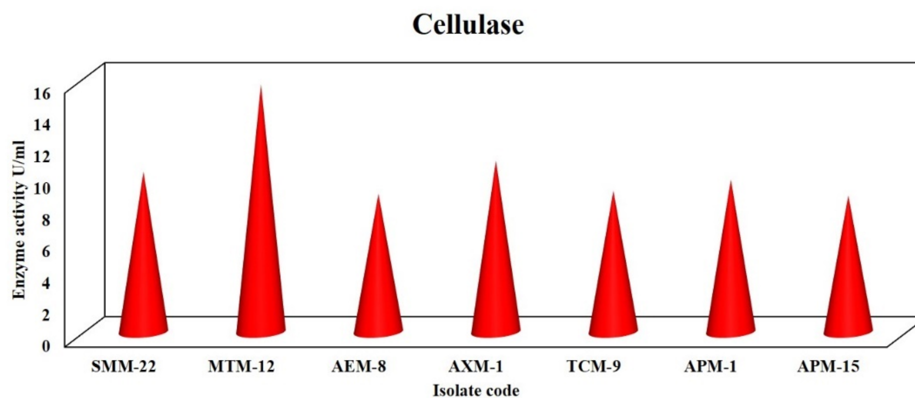


Figure: 58. Screening of filamentous moulds for their ability to produce cellulase enzyme.

Screening of Extracellular L-Asparaginase Activity

Filamentous moulds were screened for L-asparaginase production using modified Czapek Dox medium supplemented with phenol red. Change in the colour of the medium, where tested fungi are grown, from yellow to pink around colony indicated the production of asparaginase (Fig. 56c). Out of 44 filamentous moulds, 37 isolates were observed positive for extracellular L-asparaginase enzyme (Table 15). Filamentous moulds with positive response in plate screening method were screened for their quantitative production of l-asparaginase. *Aspergillus flavus (*SMM-1) isolated from *marcha* and *Aspergillus flavus* (NKM-7) isolated from *khekhrii* showed high L asparaginase activity of 8.9 U/ml and 7.28 U/ml, respectively followed by *Aspergillus versicolour* (APM-6) from *phut* (5.91 U/ml) and *Penicillium citrinum* (AEM-1) from *humao* (5.28 U/ml) (Fig. 59). Other fungal strains showed variable level of L-asparaginase activity ranging between 1.19 to 8.9 U/ml (Fig. 59).

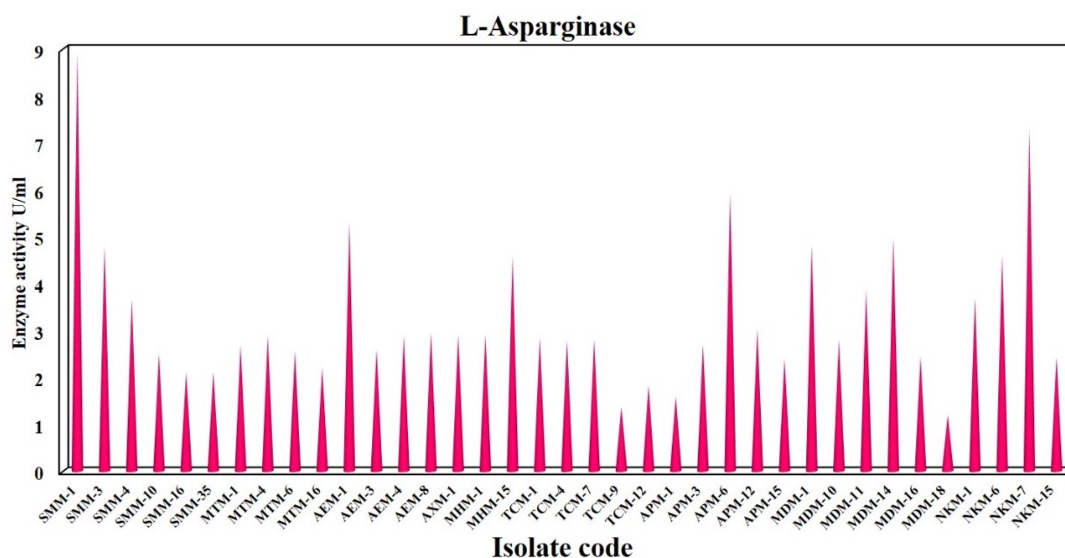


Figure: 59. Screening of filamentous moulds for their ability to produce L asparaginase enzyme.

Screening of Extracellular Lipase Activity

A qualitative phenol red agar plate method was used to estimate the lipase activity and a quantitative titrimetric assay was also performed to estimate the amount of extracellular lipase production. The cultural filtrate obtained by growing filamentous moulds was used for qualitative and quantitative estimation. Out of 44 filamentous moulds only 10 fungal strains from dry starter culture showed lipase production activity (Table 15). *Rhizopus oryzae* AEM-3 isolated from *humao* showed maximum zone of 22mm followed by *Rhizopus oryzae* (AEM-3) isolated from *chowan* and *Rhizopus oryzae* (APM-12) isolated from *phut* (Table 15).

The quantitative estimation of lipase activity was done by titrimetric method. *Rhizopus oryzae* (TCM-7) isolated from *chowan* showed highest value of lipase activity of 20.8 U/ml (Fig. 60). Other filamentous moulds *Rhizopus microsporus* SMM-4 (11.9 U/ml) isolated from *marcha* and *Rhizopus microsporus* (MDM-11) (10.9U/ml) isolated from *dawdim* showed remarkable lipase activity (Fig. 60).

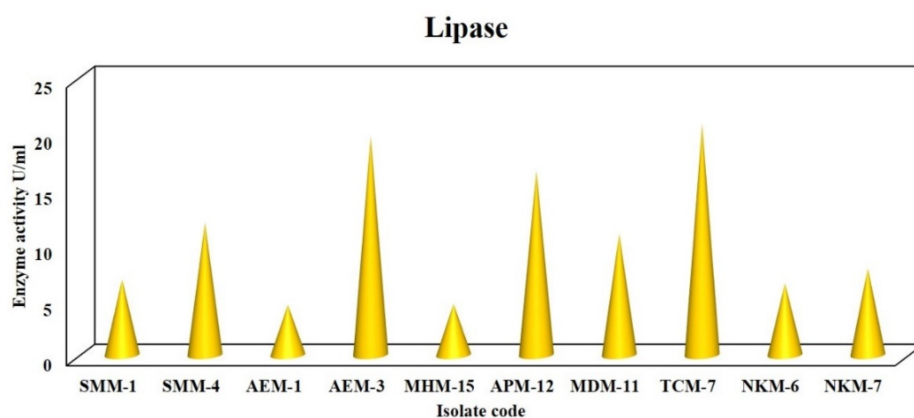


Figure: 60. Screening of filamentous moulds for their ability to produce lipase enzyme.

Screening of Extracellular Protease Activity

Screening of filamentous moulds for proteolytic activities was performed in gelatin agar medium. Out of 44 filamentous moulds screened for extracellular protease enzyme, 13 filamentous moulds were found positive for proteolytic activities based on the zone of hydrolysis (Table 15; Fig. 56e). *Aspergillus versicolour* (APM-6) isolated from *phut* showed the maximum protease activity of 54.6 U/ml (Fig. 61). *Aspergillus sydowii* (APM-1) (38.9 U/ml) isolated from *phut*, *Aspergillus flavus* (SMM-1) (38.8 U/ml) isolated from *marcha*, and *Penicillium chrysogenum* (APM-1) (38.2 U/ml) isolated from *phut* also showed remarkable protease activity (Fig. 61).

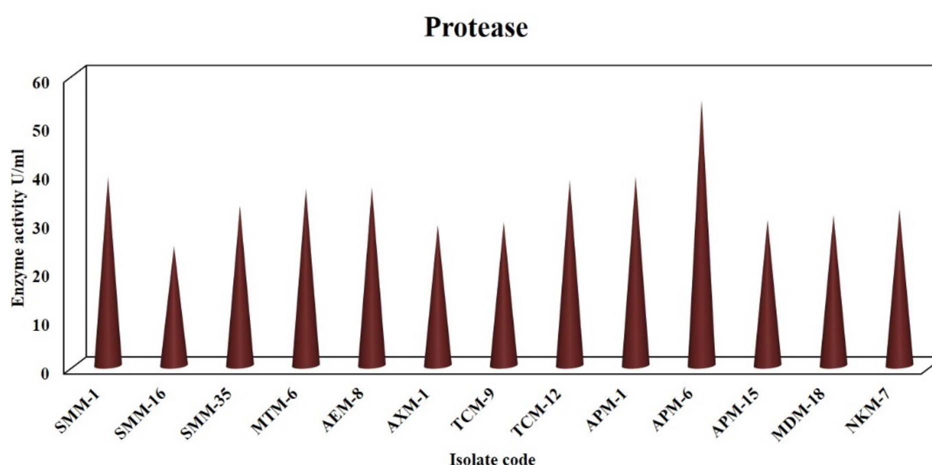


Figure: 61. Screening of filamentous moulds for their ability to produce protease enzyme.

Screening of Extracellular Xylanase Activity

Fungal strains were screened for xylanase activity by plate screening method (Table 15), however, only 16 fungal strains showed xylanase activity (Fig. 56f). Screened strains were tested for quantitative estimation of xylanase activity. *Penicillium chrysogenum* (SMM-16) isolated from *marcha* showed the higher xylanase activity of 7.8 U/ml followed by *Penicillium chrysogenum* (MDM-18) isolated from *dawdim* of 7.2 U/ml (Fig. 62). Also, *Aspergillus flavus* (SMM-1) and (NKM-7) isolated from *marcha* and *khekhrii*, showed activity of 3.5 U/ml and 3.98 U/ml, respectively (Fig 62). *Bjerkandera adusta* (MTM-16) isolated from *thiat* showed notable xylanase activity of 5.32 U/ml.

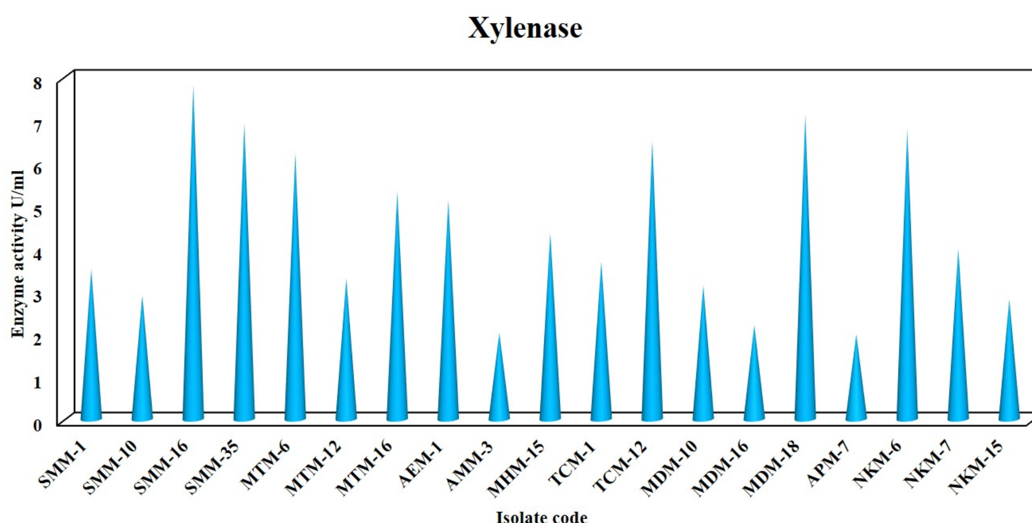


Figure: 62. Screening of filamentous moulds for their ability to produce xylanase enzyme.

SCREENING OF ANTINUTRITIVE-DEGRADING FACTOR

The screening of antinutritive degrading factor by culture plate method was performed. All 44 filamentous moulds isolated from dry starters of North East India were screened for laccase, phytase and tannase production (Table 16).

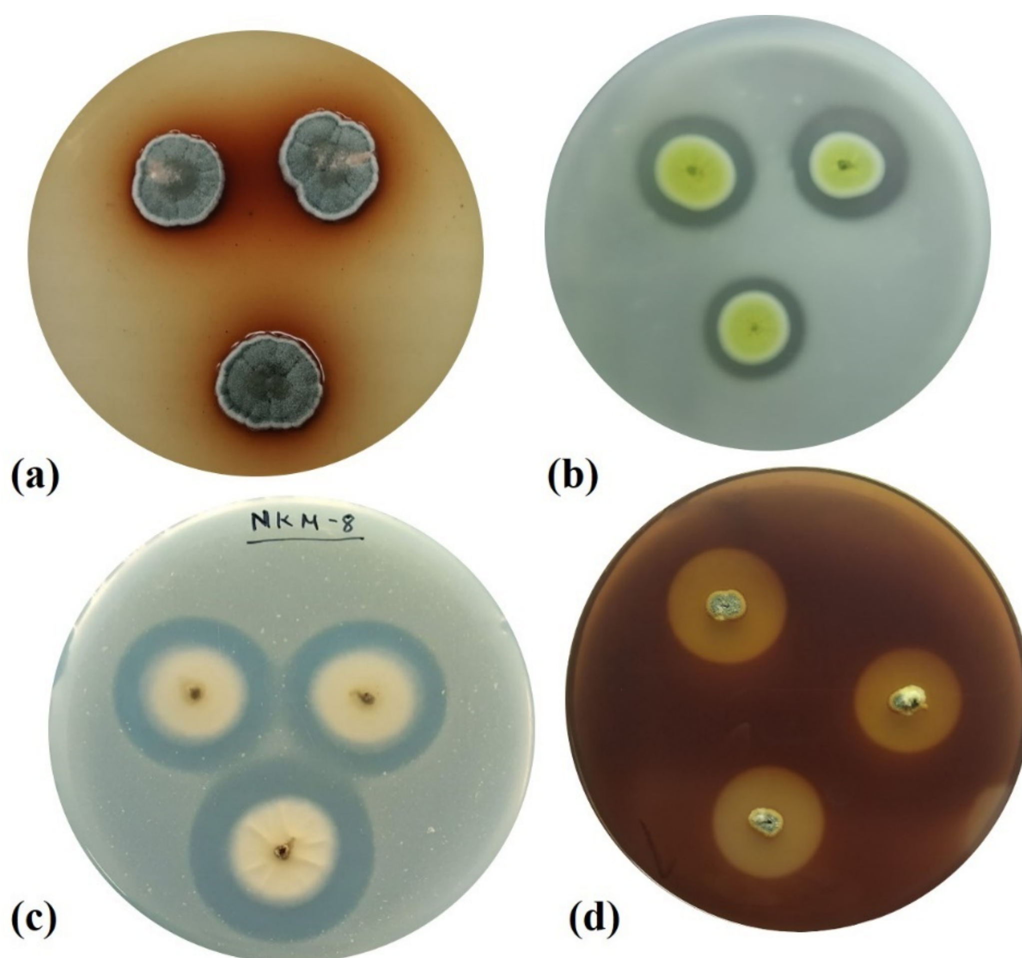


Figure: 63. (a): Plates display filamentous colonies developing reddish brown zones on 0.02% guaiacol containing medium; (**b) and (c):** Displaying filamentous moulds developing clear zone on PSM media**; (d)** Displaying the filamentous moulds developing clear zone tannin containing medium.

Table: 16. Screening of antinutritive-degrading factor of fungi isolated from dry starter of North East India

Starter	Isolate code	Fungi	Diameter (mm)					
			Laccase		Phytase		Tannase	
			Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance
Marcha	SMM-1	*Aspergillus flavus*	-	-	-	-	18	36
	SMM-3	*Mucor circinelloides*	-	-	-	-	-	-
	SMM-4	*Rhizopus microsporus*	-	-	-	-	-	-
	SMM-10	*Bjerkandera adusta*	-	-	-	-	-	-
	SMM-16	*Penicillium chrysogenum*	-	-	15	22	-	-
	SMM-22	*Penicillium polonicum*	-	-	-	-	-	-
	SMM-35	*Penicillium chrysogenum*	-	-	12	20	-	-
Thiat	MTM-1	*Mucor circinelloides*	-	-	-	-	-	-
	MTM-4	*Rhizopus delemar*	-	-	-	-	-	-
	MTM-6	*Penicillium chrysogenum*	-	-	14	22	-	-
	MTM-12	*Trametes hirsuta*	18	30	-	-	24	46
	MTM-16	*Bjerkandera adusta*	-	-	-	-	-	-
Humao	AEM-1	*Penicillium citrinum*	-	-	17	23	-	-
	AEM-3	*Rhizopus oryzae*	-	-	-	-	-	-
	AEM-4	*Mucor circinelloides*	-	-	-	-	-	-
	AEM-8	*Aspergillus sydowii*	11	20	12	24	-	-
	AXM-1	*Aspergillus sydowii*	12	23	11	22	-	-
	AMM-3	*Mucor indicus*	-	-	-	-	-	-
Hamei	MHM-1	*Mucor circinelloides*	-	-	-	-	-	-

Table: 16. Screening of antinutritive-degrading factor of fungi isolated from dry starter of North East India

Starter	Isolate code	Fungi	Diameter (mm)					
			Laccase		Phytase		Tannase	
			Fungal colony	Zone of clearance	Fungal colony	Zone of clearance	Fungal colony	Zone of clearance
	MHM-15	*Penicillium citrinum*	-	-	14	21	-	-
Chowan	TCM-1	*Bjerkandera adusta*	-	-	-	-	-	-
	TCM-4	*Mucor circinelloides*	-	-	-	-	-	-
	TCM-7	*Rhizopus oryzae*	-	-	-	-	-	-
	TCM-9	*Aspergillus sydowii*	11	21	13	28	-	-
	TCM-12	*Penicillium chrysogenum*	-	-	18	25	-	-
Phut	APM-1	*Aspergillus sydowii*	13	22	11	19	-	-
	APM-3	*Mucor circinelloides*	-	-	-	-	-	-
	APM-6	*Aspergillus versicolor*	-	-	-	-	-	-
	APM-7	*Mucor indicus*	-	-	-	-	-	-
	APM-12	*Rhizopus oryzae*	-	-	-	-	-	-
	APM-15	*Aspergillus sydowii*	14	23	12	20	-	-
Dawdim	MDM-1	*Mucor circinelloides*	-	-	-	-	-	-
	MDM-10	*Bjerkandera adusta*	-	-	-	-	-	-
	MDM-11	*Rhizopus microspores*	-	-	-	-	-	-
	MDM-14	*Mucor circinelloides*	-	-	-	-	-	-
	MDM-16	*Bjerkandera adusta*	-	-	-	-	-	-
	MDM-18	*Penicillium chrysogenum*	-	-	16	27	-	-
	NKM-1	*Mucor circinelloides*	-	-	-	-	-	-

Table: 16. Screening of antinutritive-degrading factor of fungi isolated from dry starter of North East India								
Starter	**Isolate code**	**Fungi**	**Diameter (mm)**					
			Laccase		**Phytase**		**Tannase**	
			Fungal colony	**Zone of clearance**	**Fungal colony**	**Zone of clearance**	**Fungal colony**	**Zone of clearance**
Khekhrii	NKM-6	*Penicillium citrinum*	-	-	15	29	-	-
	NKM-7	*Aspergillus flavus*	-	-	-	-	17	32
	NKM-8	*Aspergillus niger*	-	-	21	29	15	29
	NKM-10	*Penicillium oxalicum*	-	-	11	24		-
	NKM-13	*Aspergillus niger*	-	-	18	28	17	30
	NKM-15	*Cladosporium parahalotolerans*	10	22	-	-	-	-

(-) no colony of fungi and no inhibition zone

Screening of Laccase

All fungal isolates were screened for laccase production on PDA medium supplemented with 3 mM ABTS (2-2'-Azino-bis-[3-ethyl benzthiazoline-6-sulfonic acid]) and 4 mM of guaiacol as substrate (Table 16). Laccase producing isolates showed reddish brown oxidation zone in response to guaiacol, whereas showed green colour in response to ABTS. On the basis of colour of zones only 3 filamentous moulds i.e. *Aspergillus sydowii* (AEM-8), *Cladosporium parahalotolerans* (NKM-15) and *Trametes hirsuta* (MTM-12) isolated from *humao, khekhrii* and *thiat* respectively showed laccase positive result (Table 16; Fig. 63a). Filamentous moulds with positive response in plate screening method were screened for their quantitative production of laccase. In the quantifications screening of laccase, *Trametes hirsuta* (MTM-12) isolated from *thiat* showed 10.9 U/ml of laccase activity by degrading 3 mM ABTS which indicates its ability to produce laccase, anti-nutritive degrading factor (Fig. 64).

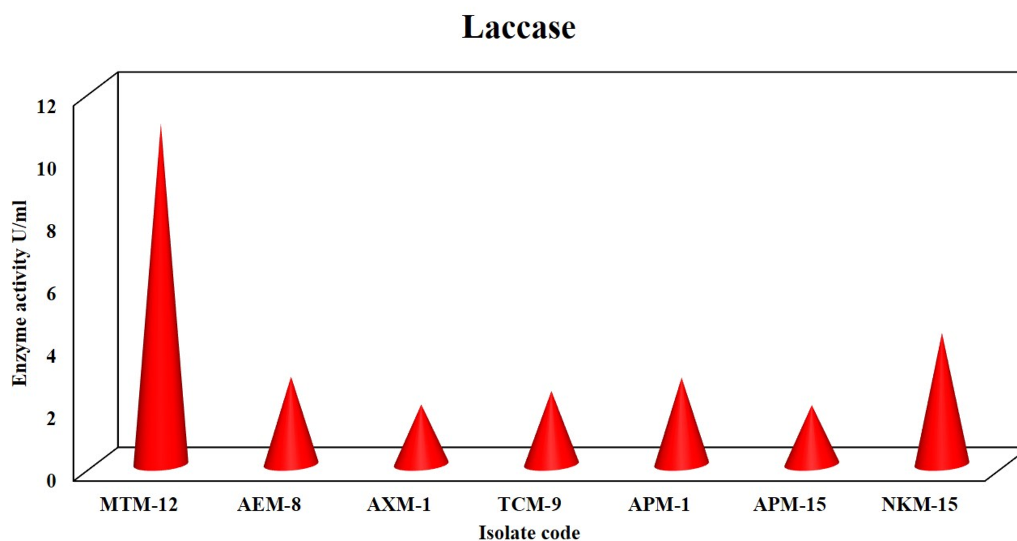


Figure: 64. Screening of filamentous moulds for their ability to produce laccase enzyme.

Screening of Phytase

The phytase producing filamentous mould isolates were screened on a medium containing sodium phytate as the sole carbon source (Table 16). Twelve filamentous colonies were picked on the basis of the clearing zones around the colonies. *Penicillium chrysogenum* (SMM-16, SMM-35, MTM-6, TCM-12 and MDM-18) isolated from *marcha, thiat, chowan* and *dawdim* respectively. *Penicillium citrinum* (AEM-1) and MHM-15 isolated from *humao* and *hamei*, *Penicillium oxalicum* (NKM-10) and *Aspergillus niger* (NKM-8 and NKM-13) isolated from *khekhrii* and *Aspergillus sydowii* (APM-1) and (APM-15) isolated from *phut*, respectively showed positive results (Table 16; Fig. 63b,c). Filamentous moulds with positive response in plate screening method were screened for their quantitative production of phytase. Among them *Aspergillus niger* (NKM-8) isolated from *khekhrii* showed highest phytase activity of 19.4 U/ml followed by *Penicillium chrysogenum* (SMM-16) (12 U/ml) isolated from *marcha* and *Penicillium chrysogenum* (MTM-6) (10.2 U/ml) isolated from *thiat* 12.0 U/ml (Fig. 65).

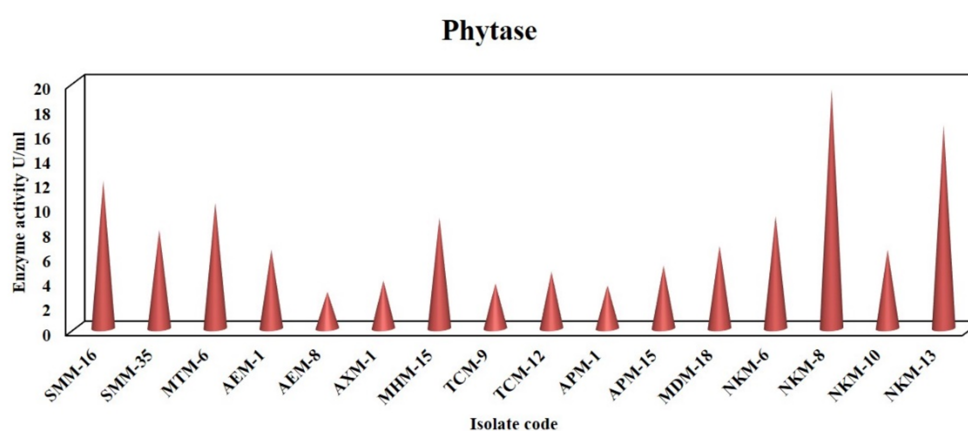


Figure: 65. Screening of filamentous moulds for their ability to produce phytase enzyme.

Screening of Tannase

Total of 44 filamentous mould isolates were screened for the production of tannase enzyme by plate assay method (Table 16). Among the all isolates only four isolates were obtained which exhibited zone of hydrolysis, thus confirming their ability to degrade tannin (Table 16). *Aspergillus flavus* (SMM-1) showed tannin hydrolysis isolated from dry starter culture of *marcha*, *Trametes hirsuta* (MTM-12) isolated from *thiat, Aspergillus flavus* (NKM-7) isolated from *khekhrii* and *Aspergillus niger* (NKM-8) and (NKM-13) isolated from *khekhrii* (Table 16; Fig. 63d). The filamentous moulds which exhibited the zone of hydrolysis on the Czapek Dox's minimal medium containing tannic acid and subjected to tannase production in Czapek Dox's minimal liquid medium for quantitative estimation of tannase activity. Five filamentous mould isolates were screened on the basis of their tannase producing efficiency under submerged condition. The isolates *Aspergillus niger* (NKM-13) isolated from *khekhrii* exhibited maximum tannase activity of 20.1 U/ml (Fig. 66), while *Aspergillus niger* (NKM-8) isolated from *khekhrii* (19.5 U/ml), *Trametes hirsuta* (MTM-12) isolated from *thiat* (12.6 U/ml), *Aspergillus flavus* (SMM-1) isolated from *marcha* (9.76 U/ml) and *Aspergillus flavus* (NKM-7) isolated from *khekhrii* (10.1 U/ml), respectively (Fig. 66).

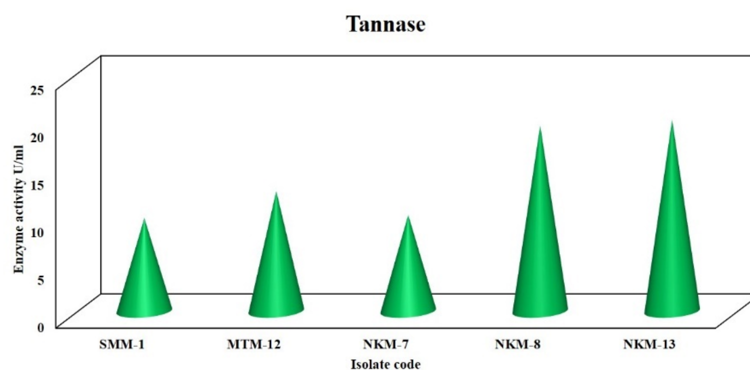


Figure: 66. Screening of filamentous moulds for their ability to produce tannase enzyme.

Selection of Fungal Species Showing Functionalities

On the basis of extracellular enzymatic activities, we selected the following fungal species isolated from traditionally prepared starters of North East India showing high functionalities, some of them were *Aspergillus niger* (NKM-8) isolated from *khekhrii* for amylase activity (27.67 U/ml), *Trametes hirsuta* (MTM-12) isolated from *thiat* for cellulase activity (15.6 U/ml), *Aspergillus flavus* (SMM-1) isolated from *marcha* for l-asparaginase activity (8.9 U/ml), *Rhizopus oryzae* (TCM-7) isolated from *chowan* for lipase activity (20.8 U/ml), *Aspergillus versicolour* (APM-6) isolated from *phut* for protease activity (54.6 U/ml) and *Penicillium chrysogenum* (SMM-16) isolated from *marcha* for xylanase activity (7.8 U/ml) (Table 17).

Table: 17. Selection of the fungal species isolated from dry starters of North East Sikkim showing highest enzymatic activity

Starter	***Enzymatic Activity (U/ml)**					
	Amylase	**Cellulase**	**L-Asparaginase**	**Lipase**	**Protease**	**Xylanase**
	Fungal species					
Marcha	*Aspergillus niger* NKM-8 (27.67 U/ml)		*Aspergillus flavus* SMM-1 (8.9 U/ml)			*Penicillium chrysogenum* SMM-16 (7.8 U/ml)
Thiat		*Trametes hirsuta* MTM-12 (15.6 U/ml)				
Chowan				*Rhizopus oryzae* TCM-7 (20.8 U/ml)		
Phut					*Aspergillus versicolour* APM-6 (54.6 U/ml)	

*Only those fungi which showed highest enzymatic activities. Though fungi isolated from *humao, hamei dawdim* and *khekhrii* showed enzymatic activities but not the highest activities.

Similarly, we selected the following fungal species isolated from traditionally prepared starters of North East India showing high anti-nutritive degrading activity, these included *Trametes hirsuta* (MTM-12) isolated from *thiat* for laccase activity (10.9 U/ml), *spergillus niger* (NKM-8) isolated from *khekhrii* for phytase activity (19.4 U/ml) and *Aspergillus niger* (NKM-13) isolated from *khekhrii* for tannase activity (20.1 U/ml) (Table 18).

Table: 18. Selection of the fungal species isolated from dry starters of North East Sikkim showing highest anti-nutritive degrading activity

	***Anti-nutritive Degrading Activity (U/ml)**		
Starter	**Laccase**	**Phytase**	**Tannase**
	Fungal species		
Thiat	*Trametes hirsuta* MTM-12 (10.9 U/ml)		
Khekhrii		*Aspergillus niger* NKM-8 (19.4 U/ml)	*Aspergillus niger* NKM-13 (20.1 U/ml)

*Only those fungi which showed highest anti-nutritive degrading activities. Though fungi isolated from *marcha, humao, hamei, chowan, phut* and *dawdim* showed anti-nutritive degrading activities but not the highest activities.

DISCUSSION

Traditional Knowledge

Drinking of cereal-based mild to strong alcoholic beverages produced by traditionally prepared amylase and alcohol-producing starter cultures is a deep-rooted dietary of the ethnic people of North East India (Tamang 2020). We documented different types of traditionally prepared starters of North East India viz. *marcha* of Sikkim, *thiat* of Meghalaya, *humao* of Assam, *hamei* of Manipur, *chowan* of Tripura, *phut* of Arunachal Pradesh, *dawdim* of Mizoram and *khekhrii* of Nagaland. During survey it was revealed that woman play a vitol role in maintaing the traditional knowledge and related skills for prepration of starter cultures. Preparation of household starter cultures require efforts and time, sometimes hardship to collect the ingredietns including wild herbs from forests, etc. Ethnobotanical knowledge for prepration of starter cultures is usually passed from mothers to daughters through oral transmission from generation to generation. We also observed that traditional starter-making trade is restricted to few families belonging to particular communities/castes/tribes. *Marcha*, a dry starter for alcohol production is preferably produced by some families belonging to Limboo and Rai of the Nepali/Gorkha community and few Lepcha families in Sikkim and Darjeeling hills, (Thapa and Tamang 2020). During interviews, a kind of jealousy about traditional recipes was perceived: most informants were able to share the general way of preparation, but not the exact dosages or similar specific details. The survey results showed that some ethnic people are engaged in preparation of starter cultures at house-hold level for home production of alcoholic beverages for commercial purposes to sell at local markets. Some people are economically dependent upon on this product. A huge part of their incomes was found to be spent for schooling of their children and to buy essential commodities whihc are not avaiable locally. Starter culture trade reflects

significant socio-economic activity in enhancing livelihood and improving social well-being of many ethnic groups of North East India (Tamang and Samuel 2010).

Starter cultures of North East India are quite similar to starters cultures of other parts of the world such as *daque* of China, *ragi* of Indonesia, *nuruk* of Korea, *manapu* and *manna* of Nepal, *bubod* of Philippines, *loogpang* of Thailand and *benh men/men* of Vietnam (Nikkuni et al. 1996; Steinkraus 1996; Lee and Fujio 1999; Nout and Aidoo 2002; Tamang and Fleet 2009, Dung et al. 2007; Jung et al. 2012; Zheng et al. 2012; Song et al. 2013; Chim et al. 2015; Tamang 2016; Daroonpuntet et al. 2016; Fronteras and Bullo 2017; Roslan et al. 2018). The traditional method of preparation of the starter culture is more or less similar, it varies from region to region in ingredients, substrate such as rice or wheat, local herbs, wrapping materials either fern or dry paddy-straw or fresh leaves of locally available wild plants, incubation periods and shape of the starter culture (Greeshma et al. 2006; Shrivastava et al. 2012; Angmo and Bhalla 2014; Tamang et al. 2016; Anupma et al. 2018). Herbs and spices are used as ingredients for making starter cultures, namely *Allium sativum, Capsicum annuum, Piper nigrum* and *Zingiber offcinale* etc. these were pound together at varying amounts (Tamang and Sarkar 1995; Tamang 2005; Shrivastava et al. 2012). These spices may source of microorganisms and may inhibit the growth of undesirable microorganisms (Hesseltine 1988; Chim, et al. 2015). Also, these spices are usually used as a food ingredient because known to help enhance the odour and taste of alcoholic beverages (Rathi 2018).

Fungal Diversity

Starter cultures of North East India were analysed for important quality attributes viz. physicochemical properties (which relates to shelf life) and filamentous moulds count which relates to safety. Moisture contents of starter cultures of North East India are low

due to sun drying process during traditional method of preparation which may increase the shelf life of the starter for a year or more at room temperature (Tsuyoshi et al. 2005; Tamang 2010). Also, low moisture is important in survival of filamentous moulds that have certain water activity requirements (Van Laarhoven et al. 2015). The pH of traditionally prepared dry starters of India were slightly acidic in nature, may be due to accumulation of metabolic organic acids (Ma et al. 2019). Moreover, low pH is favourable for the growth of mycelial fungi (Abubakar et al. 2013) and inhibitory to other microbe contaminants. The average population of filamentous moulds in traditionally prepared starter cultures of North East India was 10^5 cfu/g, which was associated with earlier reports on fungal population in *marcha* of Sikkim and Darjeeling hills in India (Tamang et al. 1988; Tamang and Sarkar 1995). No such data on fungal population in other starters of India are available except of *marcha*. Filamentous moulds, being strict aerobes are only capable of growing on the surface of starter cultures (Laranjo et al. 2019).

Traditionally prepared dry starters have consortia of co-existed microbiota containing filamentous moulds (Skory et al. 1997), yeasts (Walker and Stewart 2016) and bacteria that are crudely sub-cultured by traditional starter makers via the method of 'back-slopping" (Hesseltine et al. 1988; Tamang and Sarkar, 1995; Tamang et al. 2007; Tamang 2010b; Sha et al. 2018, 2019), for alcohol production by the Indian people. Some traditionally prepared starters of North East India have been microbiologically analysed earlier and reported several species of yeasts (Tsuyoshi et al. 1995; Jeyaram et al. 2008, 2011; Sha et al. 2017, 2018, 2019) and bacteria (Tamang et al. 2007; Pradhan and Tamang 2019). However, detailed taxonomical studies of filamentous moulds isolated from traditionally prepared dry starters of North East India have not been reported yet, except from *marcha* (Tamang et al. 1988; Tamang and Sarkar 1995;

Sha et al. 2017; 2019), *thiat* (Sha et al. 2017, 2019), *amou, perok-kushi* (Das et al. 2017). Hence, we studied the taxonomy and diversity of filamentous fungi associated with traditionally prepared dry starter cultures of North East India viz., *marcha* from Sikkim, *thiat* from Meghalaya, *humao* from Assam, *hamei* from Manipur, *chowan* from Tripura, *phut* from Arunachal Pradesh, *dawdim* from Mizoram and *khekhrii* from Nagaland based on morphological characters and molecular identifications.

In the present study, firstly we have isolated and characterised 131 fungal isolates from 40 different starters of North East India based on macroscopic and microscopic characteristics and grouped into 44 representative fungal strains. Morphological examination and identification of fungi are useful for identification up to the family or genus level (Alsohaili and Bani-Hasan 2018). However, morphological-based identification is not adequate to identify the fungi up to species level (Lutzoni et al. 2004; Tedersoo et al. 2018). Sequence-based identification tool is widely applied to confirm the exact identification of the fungal species (Romanelli et al. 2010; Xu 2016; Hofstetter et al. 2019).

We applied polymerase chain reactions (PCR) of the internal transcribed spacer (ITS) region using the primers ITS1 and ITS4 (Martin and Rygiewicz 2005) of 44 strains filamentous fungi isolated from starters of North East India and grouped into 3 phyla represented by Ascomycota (48%), Mucoromycota (38%) and Basidiomycota (14%). Similar type of phylum distribution was also reported earlier in dry starter of *nuruk* of Korea (Carroll et al. 2017) and *daqu* of China (Shoubao et al. 2019). Illustration of taxonomical keys based on morphological and molecular identification is more accurate and reliable in fungal taxonomy (Xing et al. 2018; Tedersoo et al. 2018). Hence in this Thesis we illustrated the Taxonomical Keys for filamentous moulds isolated from traditionally prepared starters of North East India based on morphological and

molecular identification, and identified 7 genera with 16 species of filamentous fungi isolated from eight starters of North East India, which were represented by *Aspergillus flavus, A. niger, A. sydowii, A. versicolor, Bjerkandera adusta, Cladosporium parahalotolerans, Mucor circinelloides, M. indicus, Penicillium chrysogenum, P. citrinum, P. oxalicum, P. polonicum, Rhizopus delemar, R. microsporus, R. oryzae* and *Trametes hirsuta*. Our earlier findings of *Rhizopus oryzae* and *Mucor circinelloides* in traditionally prepared starters of North East India by PCR-DGGE method (Sha et al. 2018) supported the present study. Hesseltine and Kurtzman (1990) reported *M. circinelloides* in *bubod* of the Philippines. *Mucor circinelloides, M. indicus, Rhizopus oryzae,* and *R. microsporus* were also reported from *benh men* of Vietnam (Dung et al. 2007; Thanh and Tuan 2008). *Aspergillus flavus* was detected in *marcha* and *khekhrii* which was also reported from *mana*, amylolytic starter of Nepal (Nikkuni et al. 1996). *Aspergillus* belonging to order Eurotials is phenotypically polythetic genus and is widely distributed in environment (Tsang et al. 2018). Samson et al. (2014) proposed phylogenetic identification of *Aspergillus* with ITS sequence data and calmodulin as a secondary identification marker, according to the decision of the International Commission of *Penicillium* and *Aspergillus* (www.aspergilluspenicillium.org). Application of ITS with β-tubulin sequences for identification of *Aspergillus* species has also been reported by Zulkifli and Zakaria (2017). However, in this study we have applied both ITS sequence and morphological characteristics, such as the conidiophore with straight ending in a large vesicle from where primary and secondary sterigmata arise bearing conidia in chains, for identification of species of *Aspergillus*. *Aspergillus niger* and *A. flavus* cannot be distinguished only by their ITS sequences, the morphological characters are also essential in species identification (Zulkifli and Zakaria 2017). We identified genus *Aspergillus* with four species in dry starters samples

of India which includes *A. niger*, *A. flavus*, *A. sydowii* and *A. versicolor*. Among *Aspergillus A. flavus, A. niger* and *A. sydowii* were most prevalent in food samples due to their sporulating abilities in the environment (Adekoya et al. 2017). *Aspergillus* is a dominant fungal genus in *daqu* of China (Ji et al. 2018) and may contribute to the saccharification process (Wang et al. 2019). We detected two strains of *A. flavus* in *marcha* sample of Sikkim (*Aspergillus flavus* SMM-1) and in *khekhrii* sample of Nagaland (*A. flavus* NKM-7). Though the distribution percentage was only 5%, the presence of *A. flavus* in samples of *marcha* and *khekhrii* is alarming. *A. flavus* is a saprotrophic with cosmopolitan distribution (Ramírez-Camejo et al. 2012) which produces aflatoxin (Saori and Keller 2011; Priyanka et al. 2012; Mudili et al. 2014). Probable source of *A. flavus* in starters may be from contaminated rice grains (Lai et al. 2015) since rice is the main base substrates for preparation of starters for production of alcohol. Moreover starter-makers commonly use low-quality, old-stocked and discarded rice grains for preparation of starters. However, due to co-existence of other species of filamentous moulds, yeasts and lactic acid bacteria in traditionally prepared starters may antagonize against *A. flavus* in *marcha* and *khekhrii,* and probably may reduce aflatoxin production in the sample (Karlovsky et al. 2016, Adebo et al. 2019). Lactic acid bacteria isolated from *marcha* showed antagonistic property (Tamang et al. 2007), similarly some bacteria have antifungal activity against aflatoxin-producing *A. flavus* (Shakeel et al. 2018). *Rhizopus* spp. from *tempeh,* fermented soybean food of Indonesia, were reported for detoxification of alfatoxin (Nakazato et al. 1990). *A. sydowii* present in samples *humao*, *phut* and *chowan*, is an industrially important filamentous mould, which produces monosaccharides and indole alkaloids (Zhou et al. 2018). None of the starters of North East India showed the presence of *A. versicolor* except in *phut* samples of Arunachal Pradesh. *A. versicolor* is a slow-growing

filamentous fungus commonly found in/on damp indoor environments (Samson et al. 2004), foods and feeds (Jurjevic et al. 2012) and produces toxic metabolites (Piontek et al. 2016). Contamination of *A. versicolor* in *phut* samples might be from the damp room where preparation of *phut* is often practiced by starter producer in Arunachal Pradesh. By ITS sequencing tool, it will be difficult to distinguish among the different species of *Mucor circinelloides* complex (MCC) which include *M. circinelloides, M. griseocyanus, M. janssenii, M. lusitanicus, M. ramosissimus, M. variicolumellatus* and *M. velutinosus* (Wagner et al. 2019). Hence we used species from the *Mucor circinelloides* complex. Species of *Mucor circinelloides* complex (MCC) were found the most dominant fungi in dry starter cultures of North East India. *M. circinelloides* complex has sub-globose sporangiospore with sympodial branching pattern. *Mucor circinelloides* complex contributes in saccharification and liquefaction of cereal during fermentation of *kodo ko jaanr*, an alcoholic product of Sikkim fermented by starter *marcha* (Thapa and Tamang, 2004; Tamang and Thapa 2006). *M. circinelloides* is an oleaginous fungus (Qiao et al. 2018) which produces lipids (Wei et al. 2013), cellulose degrading enzyme (Huang et al. 2014) and has several functional properties including antioxidants (Hameed et al. 2017). Phylum Mucoromycota does not produce mycotoxins, however, some species belong to this group *M. circincelloides* forma *circinelloides* has been described to be putatively responsible for human illnesses after consumption of mould contaminated yoghurt (Lee et al. 2014) although its involvement was not clearly proven. *M. circinelloides* was also earlier reported in *marcha* samples (Tamang et al. 1988; Tamang and Sarkar 1995). *M. indicus*, isolated from *humao* of Assam and *phut* of Arunachal Pradesh, is a dimorphic and ethanolic fungus which is able to produce ethanol from glucose, mannose, fructose and galactose (Karimi and Zamani 2013) and oil, protein, and glucosamine (Sharifyazd and Karimi 2017).

Phylogenomic studies show that genus *Rhizopus* has three major clades viz. *R. microsporus with* its sister taxon *R. stolonifer*, *R. arrhizus* and *R. delemar* (Gryganskyi et al. 2018). *Rhizopus oryzae,* commonly present in soils, animal excrement and rotting vegetables (Ghosh and Ray 2011) is very similar to *Rhizopus stolonifer*, except its smaller sporangia with air-dispersed sporangiospores (Pitt and Hocking 2009). *R. oryzae* and *R. microsporus* are detected in *yao qu* of China and *banh men* of Vietnam, which are strong amylase producers (Dung et al. 2007; Thanh et al. 2008; Lv et al. 2012b). *R. oryzae* is considered as a GRAS (generally regarded as safe) filamentous fungus (Londoño-Hernández et al. 2017), which is commonly used for production of some Asian fermented foods (Tamang et al. 2016a). *Rhizopus microsporus* is the major fungus in *tempe*, fermented soybean food Indonesia (Hartanti et al. 2015). *R. delemar* was found in *thiat* sample only, which naturally accumulates fumaric acid with fruity taste (Odoni et al. 2017), probably it imparts taste and flavour in *kiad,* an alcoholic product fermented by starter *thiat. R. delemar* has also been reported in *xajpitha,* starter of Assam in India (Bora et al. 2016). Presence of *Rhizopus* spp. in starters of North East India may contribute functional properties in end products during acholic fermentation (Tamang et al. 2016b). *Penicillium chrysogenum* was found only in four starters viz. *marcha* (Sikkim), *thiat* (Meghalaya)*, chowan* (Tripura) and *dawdim* (Mizoram). The probable entry of *P. chrysogenum* during traditional preparation may be from damped and moist rooms where usually preparation for such starters is being done, since *P. chrysogenum* is found in damp building (Andersen et al. 2011). Due to ability of *P. chrysogenum* to produce antibiotics mostly penicillin (Bajaj et al. 2014), its presence in starters may have antagonist property in the end product. *P. citrinum* was recovered in samples of *humao, hamei* and *khekhrii*, probable through indoor environments (Samson et al. 2004). *P. oxalicum* was found in sample of *khekhrii* (Nagaland) and *P. polonicum*

in *marcha* sample. *P. oxalicum* produces various enzymes and natural products (Li et al. 2016). *P. polonicum* has been reported in fermented black table olives (Bavaro et al. 2017). Interesting we detected *Bjerkandera adusta* (Basidiomycetous fungus) in *marcha, thiat, dawdim, chowan* and also *Trametes hirsuta* (Basidiomycetous fungus) in *thiat*. *B. adusta* and *T. hirsuta* are wood decaying white-rot fungi (Rosales et al. 2005; Horisawa et al. 2019). *B. adusta* grows on a natural cellulosic substrate, imparts refreshing aroma (Zhang et al. 2015), contributes in saccharification (Quiroz-Castañeda et al. 2009) and produces ethanol (Horisawa et al. 2019). *Trametes hirsuta* is lignin degrading fungus due its ability to synthesis laccase (Cilerdzic et al. 2011). Traditional method of preparation of these amylolytic starter cultures require locally grown wild plants and spices used as ingredients by local starter-producers (Anupma et al. 2018). We assume that during collection of wild plants from forest grounds, people might have collected whole wild plants *in situ*, where wood-rooting fungi have been reported in forest of North East India (Chuzho et al. 2017). There is no practice of filtering and cleaning of collected wild plants during starter preparation, hence chance of contamination of these basidiomycetous fungi may be possible during preparation. *B. adusta* and *T. hirsuta* are not reported earlier in any starter culture or in any fermented food. *Cladosporium parahalotolerans* was found only in samples of *khekhrii*. *C. parahalotolerans* is mostly occurred in plant debris, foods and indoors (Bensch et al. 2012). Source of *Cladosporium* in *khekhrii* might be from wild herbs used as ingredients during traditional preparation of *khekhrii* in Nagaland. Species of *Bjerkandera, Trametes* and *Cladosporium* have not been reported in any fermented foods elsewhere.

Diversity indexes determine the phylogenetic relations within different fungal species in a community (Fernandes et al. 2015). We calculated diversity indexes of fungal

community present in starters of North East India by Shannon's diversity index (H), species evenness (E) and species richness (R). Shannon diversity index (H) for evaluating fungal diversity was recorded highest in *marcha* samples collected from Sikkim (H: 1.74) and lowest in *hamei* samples of Manipur (H: 0.69) indicating higher fungal diversity in *marcha* samples of Sikkim as compared to starters of other states. Diversity index which considers both numbers of species as well as relative abundance of each species for evaluating diversity (Lucas et al. 2017), showed highest value for *marcha* of Sikkim. Species richness is the number of different species represented in an ecological community, where it does reflect the abundances of the species or their distributions (Unterseher et al. 2008). Species Richness (R) values in samples of *marcha* and *khekhrii* were recorded highest showing its more diversity in species level of filamentous moulds. Species evenness refers to how equal the community is numerically ranging from 0 to 1 value (Savary et al. 2018) signifying the value 1.0 in *thiat, hamei* and *chowan* a complete evenness comparable to other starters. Hence diversity index of filamentous fungal community present in dry starters of North East India showed high diversity within the community. It was observed that there was variation in fungal species distribution in each type of dry starters in North East India which may determine the quality of the acholic product preferred by the local consumers. This might be due to variable geographical regions, biotic and abiotic factors and different plant species/spices used in the methods of preparation of dry starters. Hence it shows that fungal diversity present in amylase and alcohol-producing starters, traditionally prepared by ethnic Indian people using their traditional knowledge of “back-slopping”, are morphologically, ecologically and phylogenetically diverse. Findings on fungal diversity in dry starters of North East India may supplement the

microbial diversity in eco-systems of North East India, which is one of the biodiversity hot spots of the world (Chettri et al. 2010).

Fungal Community Structure in *Marcha* and *Thiat*

We selected two dry starters viz. *marcha* of Sikkim and *thiat* of Meghalaya for studies on fungal community structures using high-throughput sequencing tool. Quantitative differences were reported for the presence of fungal community between *marcha* and *thiat* which may be the result of variations in preparation process, time of incubation and most importantly, type of preservations. Shannon index indicated less fungal diversity in *thiat* while *marcha* displayed a higher assemblage of fungal diversity with dominance of Ascomycota yeast phylum. The existence of higher fungal diversity in *marcha* is a determinant of the higher acidic conditions of *marcha*; less fungal diversity of *thiat*, on the other side, indicates the faster transition from acid to alkaline with acid presence (Sha et al. 2017). Investigation of fungal diversity of dry starters showed higher abundance of yeast in *marcha* and *thiat* than that of filamentous moulds. In *marcha,* no filamentous moulds were detected by high throughout sequencing methods, the reason behind that is limited sample size, age of sample size, lower abundance of filamentous moulds diversity and finally due to insufficient cell lysis that may not allow nucleases to be released (Dolci et al. 2015; Sha et al. 2017; 2019). Analysis of *thiat* starter culture of Meghalaya has revealed the presence of *Aspergillus penicillioides*, *Aureobasidium pullulans* and *Mucor circinelloides* as the most prevalent filamentous moulds. The fungal diversity of *xaj-pitha*, Assam starter cultures, was investigated by using a next-generation sequencing approach involving Illumine platform-based whole genome shotgun sequencing method and revealed the existence of producers of amylase such as *Rhizopus delemar, Mucor circinelloides, Aspergillus* species (Bora et al. 2016).

The Phyla level distribution showed *Ascomycota* and *Zygomycota* phyla were dominant phyla in *marcha* and *thiat*. Similarly, dominant phyla *Zygomycota* in Korean *nuruk,* although *Ascomycota* was present in fewer numbers (Bal et al. 2016). These phyla were claimed to be the key phyla during fermentation of rice beer (Jung et al. 2012).

Enzymatic Activities

Temperature is one of the factors that influences fungal growth (Li et al. 2009; Agusti-Brisach and Armengol 2012; Ali et al. 2017). In the present study, we found that *Aspergillus flavus, A. niger,* and *A. sydowii* grew at temperatures ranging between 20-40ºC but optimally *Bjerkandera adusta, Penicillium chrysogenum, Penicillium citrinum Penicillium polonicum* and *Trametes hirsute* grew well at 20ºC to 30ºC. Within this temperature range, these filamentous moulds produce a typical morphology and good spore production. Filamentous moulds can live in a relatively wide range of temperatures, but their growth rate and metabolism differ at different temperatures even when other conditions are constant, such as kinds of nutrient, water activity and many other environmental factors (Carlile et al. 2001; Burge 2006). The temperature at which a mould has the highest rate of increase in biomass is normally accepted as the optimum temperature (Kamil et al. 2011). As temperatures exceed the optimum temperature, chemical reactions occur less efficiently, and growth slows (Burge 2006; Ali et al. 2017). Several researchers reported a different behaviour of the *A. flavus* isolated with values ranging from 25 to 30ºC regardless of the media (Lahouar et al. 2016). Singh and Chauhan 2013 reported that the most suitable temperature for the growth of *Aspergillus flavus* and *Penicillium. chrysogenum* was observed on 25°C and 30°C, respectively. *A. niger*, has its own distinct growth character, particular at temperatures above 35ºC. While *A. niger* grew extremely well in all media at 41ºC (Palacios-Cabrera

et al. 2005). *Mucor indicus*, *Mucor circinelloides*, *Rhizopus delemar, Rhizopus microspores* and *Rhizopus oryzae* attained colony diameter between 70-80 mm at their optimum temperatures i.e. 30ºC. *Mucor* and *Rhizopus* are very susceptible to temperature which affect the viability of sporangiospores (Dennis and Blijham 1980). The temperature has a considerable effect on growth of wood-rotting fungi it varies from species to species i.e. *Bjerkandera adusta* and *Trametes hirsuta* (10-40ºC). We observed that growth rates of filamentous moulds in starters were affected by different temperatures, which may lead to significant change in the microbial community adapting to the new condition (Pietikäinen et al. 2005).

Filamentous isolates were screened for enzyme production such as amylase, cellulase, L-asparaginase, lipase, protease and xylanase. Amylases are of great significance for starch hydrolysis (conversion of polysaccharides to monosaccharides) and other associated oligosaccharides in the food fermentation and food industries (Geisen 1993; Gopinath et al. 2017). Cellulase enzyme help to turn biomass into ethanol by fermenting the sugars (McKelvey and Murphy 2011). Fungal protease has been used to hydrolyze proteins that cause turbidity in alcoholic beverages (Mamo and Assefa 2018). Several isolates revealed the presence of cellulase, L-asparaginase, lipase, protease and xylanase activity that could improve the texture aromatic profile of dry starter culture and can be interest for major industrial applications (Raveendran et al. 2018). The occurrence of amylase producing organism from dry starter culture of North East India agrees with earlier report that starter cultures are known to be source of amylase producer (Tamang et al. 2016b). Various amylase producing microorganisms have been reported earlier (El-Fallal et al. 2012; Fadahunsi and Garuba 2012; Gopinath et al. 2017; Martin et al. 2019). *A. flavus* (SMM-1) shows 22.06 Um/L at 30ºC (Geetha et al. 2011; Fadahunsi and Garuba 2012). According to Singh and Gupta (2014) *A. flavus* TF-8

shows 26.38 U/ml; amylase activity under submerged fermentation. Also, three species of *Penicillium* (*P. chrysogenum*, *P. citrinum* and *P. oxalicum*) were also capable of secreting amylase. Saleem and Ebrahim (2014) reported that *A. flavus*, *A. sydowii*, *As. versicolor, M. circinelloides, P. chrysogenum*, *P. citrinum* and *P. oxalicum* were amylase producers.

Cellulase is an important enzyme that assists the degradation of cellulose (Ram et al. 2014) and is responsible for the cleavage of the β–1, 4–glycosidic linkages in cellulose (Payne et al. 2015). Cellulose can be considered the most abundant and biologically sustainable resource for its conversion, its use of glucose and other soluble sugars for use as liquid fuel can be maximized (Jahangeer et al. 2005; Ram et al. 2014). Cellulases enzyme has great potential for saccharification and fermentation also for increasing process performance and yield, improving the extraction methods, clarification and stabilization of juice (Kumar et al. 2019). Cellulase activity of the filamentous moulds was evaluated by cellulase assay, where cellulase was liberated in CMC solution through DNS method (3, 5-dinitrosalicylic acid) and the activity of the enzyme was expressed in U/ml (Yuan et al. 2012; Akula and Golla 2018). The cellulase activity of *Trametes hirsuta* (MTM-12) was 15.6 U/ml. *T. hirsuta* is a strong ligninolytic, cellulolytic filamentous moulds (Jeya et al. 2009). *Aspergillus* produces a wide range of enzymes which can degrade polysaccharides in the plant cell wall. In present study most of the cellulase producers identified belonged to genera *A. sydowii* (AEM-8, AXM-1, TCM-9, APM-1 and APM-15) (Verma and Verma 2016) and *P. polonicum* (SMM-22). Khokhar et al. (2011) showed that *Trichoderm, Aspergillus* and *Penicillium* were isolated from different sources, screened and compared for their ability to degrade cellulose.

Filamentous moulds were screened for L-asparaginase production using modified Czapek Dox medium supplemented with phenol red (Doriya and Kumar 2016), where colour changes from yellow to pink around colony due to change in medium pH, as L-asparaginase causes amide bond breakdown in L-asparagine and liberated ammonia (Vaishali and Bhupendra 2017). L-asparaginase was found in *Mucor circinelloides* (SMM-3, MTM-1 AEM-4, MHM-1, TCM-4, APM-3, MDM-1, MDM-14 and NKM-1)*,* followed by *Aspergillus flavus* (SMM-1 and NKM-7)*, Rhizopus delemar* (MTM-4)*, Rhizopus microsporus* (SMM-4 and MDM-11)*, Rhizopus oryzae* (SMM-1, AEM-3, TCM-7 and APM-12)*, Penicillium chrysogenum* (SMM-16, SMM-35, MTM-6 and TCM-12)*, Penicillium citrinum* (AEM-1, MHM-15 and NKM-6); but on other hand *Aspergillus sydowii* (AEM-8, AXM-1, TCM-9, APM-1, APM-15 and MDM-10)*, Aspergillus versicolor* (APM-6)*, Bjerkandera adusta* (SMM-10, MTM-16, TCM-1 and MDM-16*. Cladosporium parahalotolerans* (NKM-15) was the slowest L-asparaginase producers among all selected positive isolates. Earlier report suggested that *Aspergillus* and *Penicillium* have important source of this enzyme (Sarquis et al. 2004; Cachumba et al. 2016). These observations were partially in agreement with those previously reported for L-asparaginase activity from *A. flavus* (9.88 U/ml), *A. versicolor* (6.70 U/ml) *Penicillium citrinum* (6.11 U/ml) and *Rhizopus oryzae* (2.01 U/ml) (Patro et al. 2014; Bedaiwy et al. 2016).

Extracellular lipase secreted by filamentous moulds is widely used in the food industry (Mehta et al. 2017; Kumar and Ray 2014). Assays using agar plates are conducted because lipase activities are difficult to determine because of the water-soluble enzyme that acts on insoluble substrates (Griebeler et al. 2011; Gopinath et al. 2013). In the present work filamentous mould *Aspergillus flavus* (SMM-1 and NKM-7) from *marcha* and *khekhrii, Rhizopus oryzae* (AEM-3; TCM-7; APM-12) isolated from starter culture

humao, chowan and *phut, Rhizopus microspores* (SMM-4 and MDM-11) isolated from *marcha* and *khekhrii*, *Penicillium citrinum* (AEM-1, MHM-15 and NKM-6) from *humao, hamei* and *khekhrii* showed lipolytic activities. Extracellular lipase production by *Aspergillus* sp, *Fusarium* sp., *Penicillium* sp., *Rhizopus* sp. were also reported earlier (Colen et al. 2006; Parveen and Manikandaselvi 2011; Ayinla et al. 2017; El-Ghonemy et al. 2017; Ramnath et al. 2017).

Protease have been the most widely studied enzyme because that it has a wide range of application in food industry (Tavano et al. 2018; Razzaq et al. 2019). Protease helps improve the nutritional and functional value of food, the digestibility and sensory quality of food, as well as provide health benefits (Souza et al. 2015; Şanlier et al. 2019). It plays a significant role in the production processes of the food industry, particularly in cheese, meat, fish, bakery, brewing and fermented food (Razzaq et al. 2019; Tamang et al. 2020). Proteases improve the taste of the products (Okpara et al. 2019). In the present study, filamentous moulds from starter cultures were primary screened for extracellular protease activity. Maximum activity of protease was recorded in *A. versicolour* (APM-6) from *phut*, which was also reported in *A. versicolour* (Hossain et al. 2006; Choudhary and Jain 2012) in *Aspergillus flavus* (Oyeleke et al. 2010 and Hossain et al. 2006), and in *Aspergillus sydowii* (Palaniswamy et al. 2017). In line with the present study *Penicillium chrysogenum* produced the maximum protease production 12.79 U/ml after 72 h of incubation (Ikram-Ul-haq and Umber 2006). In this study we determine the xylanase activities of filamentous moulds by qualitative plate screening and the quantitative fermentation techniques. Xylanases also called xylosyl hydrolases are important in food and feed industry and brewing industry (Collins et al. 2005; Bhardwaj et al. 2019). Nineteen filamentous moulds from starter culture showed xylanolytic potential as examined based on Congo red staining with 1M

NaCl., out of which *Mucor indicus* (AMM-3) showed the highest xylanase activity. Terrone et al. (2018) reported that *Penicillium chrysogenum* F-15 strain showed highest xylanase production.

Antinutritive-degrading Factor

This study showed among 44 strains of filamentous moulds, only three strains were screened positive for laccase enzyme using 3 mM of ABTS and 4 mM of guaiacol (Senthivelna et al. 2019). We also checked the enzyme activity and selected *Trametes hirsuta* (MTM-12) isolated from *thiat* a potential strain for laccase production (Rodríguez Couto et al. 2006), which plays a role in wood decomposition as producer of laccase (Tomšovský and Homolka 2003; Glazunova et al. 2018).

Phytase is widely used to act on phytic acid for the aim of increasing the bioavailability of phosphorus, proteins and essential minerals during fermentation process of alcoholic beverage (Vashishth et al. 2017). It is commercially produced by employing a submerged fermentation process using spore inoculum of filamentous moulds (Shah et al. 2017). In the present study, we selected 12 filamentous moulds on the basis of formation of zone around the colony. According to study *Aspergillus niger* (NKM-8) showed the best activity of phytase (19.4 U/ml). *Penicillium chrysogenum* were also potential source of phytase. Phytase activities are shown by *Aspergillus niger* (Bhavsar et al. 2013), *A. Japonicus* (Moreira et al. 2014), *Rhizopus oryzae* (Ramachandran et al. 2005) and *Penicillium chrysogenum* (Pires et al. 2019).

Tannase from *Aspergillus flavus* was employed to hydrolyze the polyphenols in alcoholic beverages and also for flavour development (Kumar et al. 2019a). Some filamentous moulds were found to produce tannase in dry starter culture of North East India, among which *Aspergillus niger* NKM-13 showed the highest activity. Similarly, Girdhari and Peshwe (2015) reported the production of tannase by *Aspergillus niger,*

niger, *A. flavus* and *A. oryzae* were found to be the best tannase producers on tannic acid as a sole source of carbon (Yamada et al. 1968). Several filamentous moulds are known to be tannase producers such as *Aspergillus, Fusarium, Paecilomyces, Penicillium, Neurospora, Trichothecium, Trichoderma*, genera (Zakipour-Molkabadi et al. 2013; Herrera Bravo de Laguna et al. 2015; Prigione et al. 2018).

CONCLUSION

We studied the taxonomy of filamentous moulds and their diversity in dry starter culture of North East India (*marcha*, *thiat, humao*, *hamei, chowan*, *phut*, *dawdim* and *khekhrii*) and also their enzymatic and antinutritive degrading activities. Based on illustrated taxonomical keys, 7 genera with 16 species of filamentous moulds from identified viz. *Aspergillus flavus, Aspergillus niger*, *Aspergillus sydowii*, *Aspergillus versicolor*, *Bjerkandera adusta*, *Cladosporium parahalotolerans*, *Mucor circinelloides*, *Mucor indicus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium oxalicum*, *Penicillium polonicum, Rhizopus delemar, Rhizopus microsporus, Rhizopus oryzae* and *Trametes hirsuta*. We also analysed starter culture of Sikkim *marcha* and Meghalaya *thiat* samples using high-throughput sequencing tool. Some filamentous moulds showed high activities of some enzymes such as amylase, l-asparaginase, lipase, protease and xylanase. Even some of them showed antinutritive degrading ability. Filamentous moulds in traditionally prepared starters of North East India have ethnical, socio-cultural, microbiological and functional properties.

SUMMARY

Mild-alcoholic beverages are commonly prepared from cereals in North East India by some ethnic people by using traditionally prepared dry, round to flat shaped and variable sizes starter cultures through "back-slopping" methods for centuries. Amylase- and alcohol-producing starters are consortia of filamentous moulds, yeasts and bacteria. Yeasts and bacteria present in these starters of North East have already been reported earlier. However, no information in respect of fungal communities and their diversity in dry starters are available, except in few. Filamentous moulds are important organisms in traditionally prepared dry starters of North East India. The major objectives of this thesis were to document indigenous knowledge of people of North East India on production of traditionally prepared non-food amylolytic and alcohol producing starters in the form of dry, solid, oval-flat cake-like starters viz. *marcha* of Sikkim, *thiat* of Meghalaya, *humao* of Assam, *hamei* of Manipur, *chowan* of Tripura, *phut* of Arunachal Pradesh, *dawdim* of Mizoram and *khekhrii* of Nagaland and to investigate the filamentous moulds diversities. Also, to estimate the extracellular enzymatic activities and antinutritive degrading factor activities of the identified filamentous mould isolates. The average filamentous mould population was 4.9 x 10^5 cfu/g with average moisture 10.7 % and pH 5.3, respectively. In present study, 131 filamentous mould isolates were isolated and characterised based on macroscopic as well as microscopic characteristics and grouped into 44 representative fungal strains. Based on the results of morphological characteristics and ITS gene sequencing, 44 fungal strains were grouped into 3 phyla represented by Ascomycota (48%), Mucoromycota (38%) and Basidiomycetes (14%). Taxonomical keys to species level were illustrated on the basis of morphological characteristics and ITS gene sequencing aligned to fungal database of NCBI GenBank which showed 7 genera with 16 species represented by *Mucor circinelloides* (20%), *Aspergillus sydowii* (11%), *Penicillium chrysogenum* (11%), *Bjerkandera adusta*

(11%), *Penicillium citrinum* (7%), *Rhizopus oryzae* (7%), *Aspergillus niger* (5%), *Aspergillus flavus* (5%), *Mucor indicus* (5%) *Rhizopus microsporus* (5%), *Rhizopus delemar* (2%), *Aspergillus versicolor* (2%), *Penicillium oxalicum* (2%), *Penicillium polonicum* (2%), *Trametes hirsuta* (2%) and *Cladosporium parahalotolerans* (2%). Strangely, we recognized few Basidiomycota filamentous moulds represented by *Bjerkandera adusta* and *Trametes hirsuta* in *marcha, thiat, chowan* and *dawdim* samples. The Shannon diversity index (H) was recorded highest in marcha of Sikkim (H: 1.74) and lowest in hamei of Manipur (H: 0.69). Fungal species present in these dry starters are morphologically, ecologically and phylogenetically diverse and showed high diversity among the community.

We additionally studied two samples of North East India randomly i.e. *marcha* of Sikkim and *thiat* of Meghalaya by using high-throughput amplicon sequencing method for complete profiling of fungal communities. The raw sequencing data showed the higher diversity of filamentous moulds in *thiat* whereas *march*a showed the higher yeasts diversity. The ITS sequencing and taxonomic raw data analysis revealed the predominance of yeast phylum Ascomycota (98.6%) in *thiat*, whereas it was only 1.4% in phylum Mucoromycota. However, in *marcha* only yeast phylum Ascomycota constituted the 100 % of fungal diversity. Distributions of fungi at the family level in *thiat* were *Trichocomaceae* (15.7%), *Dothioraceae* (3.94%), *Mucoraceae* (2.63%) and unidentified fungi (77.73%). The sequence reading revealed that yeast community dominated the fungal composition in *marcha* while the fungal composition in *thiat* was dominated by filamentous moulds.

In the present study, we checked the effect of temperature on fungal growth and found that *Aspergillus flavus, A. niger,* and *A. sydowii* grew at temperatures ranging between

20-40ºC but optimally *Bjerkandera adusta, Penicillium chrysogenum, Penicillium citrinum Penicillium polonicum* and *Trametes hirsute* grew well at 20 to 30ºC.

Filamentous moulds isolated from traditionally prepared starters of North East India were screened for extracellular enzymes production such as amylase, cellulase, L-asparaginase, lipase, protease and xylanase. Out of 44 filamentous moulds, *Aspergillus niger* (NKM-8) isolated from *khekhrii* showed highest amylase activity (27.67 U/ml), *Trametes hirsuta* (MTM-12) isolated from *thiat* for cellulase activity (15.6 U/ml), *Aspergillus flavus* (SMM-1) isolated from *marcha* for l-asparaginase activity (8.9 U/ml), *Rhizopus oryzae* (TCM-7) isolated from *chowan* for lipase activity (20.8 U/ml), *Aspergillus versicolour* (APM-6) isolated from *phut* for protease activity (54.6 U/ml) and *Penicillium chrysogenum* (SMM-16) isolated from *marcha* for xylanase activity (7.8 U/ml).

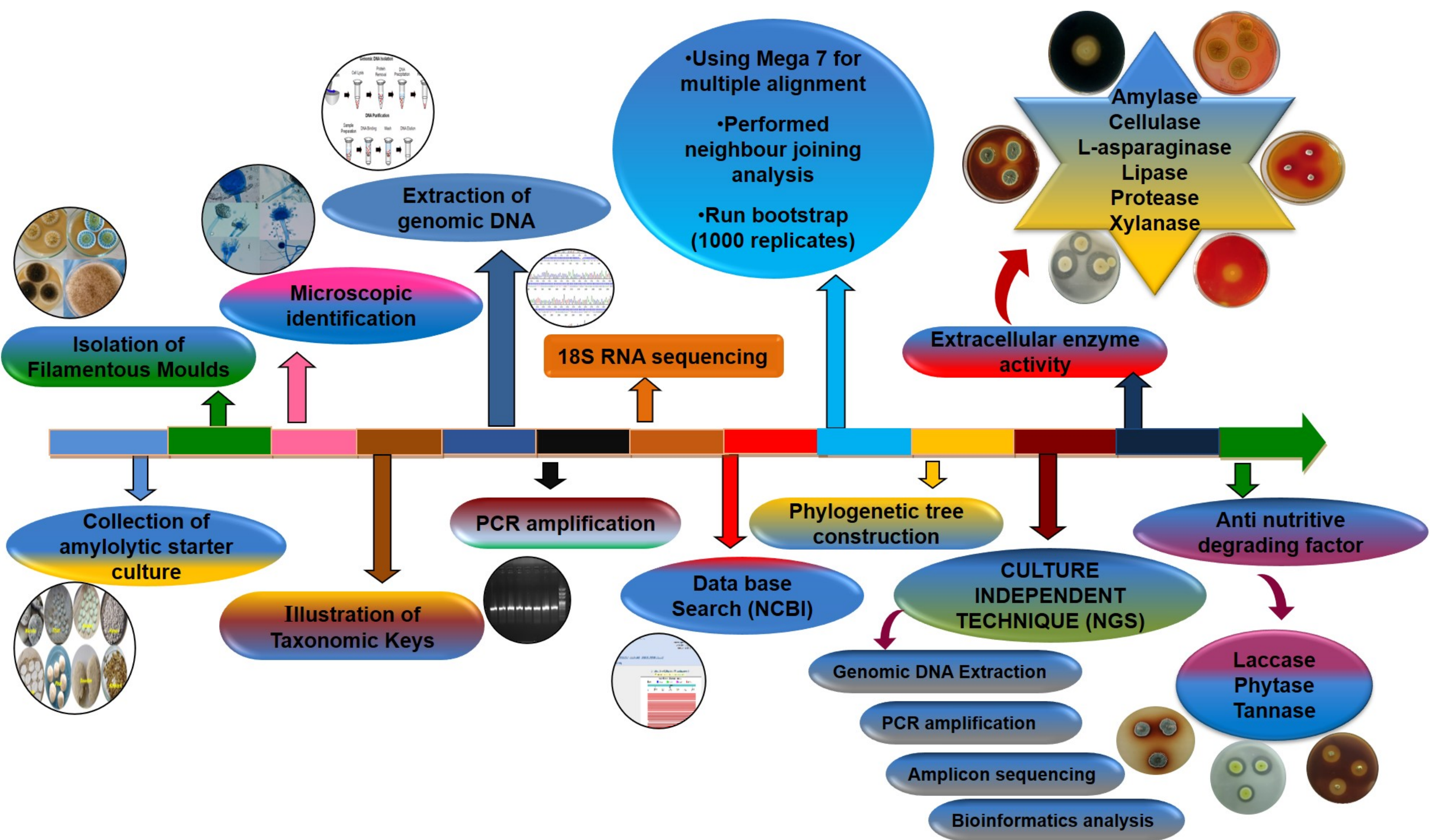
We also screened 44 filamentous moulds for anti-nutritive degrading activities, out of which, *Trametes hirsuta* (MTM-12) isolated from *thiat* showed high laccase activity (10.9 U/ml), *Aspergillus niger* (NKM-8) isolated from *khekhrii* showed high phytase activity (19.4 U/ml) and *Aspergillus niger* (NKM-13) isolated from *khekhrii* showed high tannase activity (20.1 U/ml), respectively.

Findings revealed that traditional dry starter cultures of North East India are microbiologically composed of diverse species of filamentous moulds which have several enzymatic and anti-nutritive degrading activities, and may be explored for industrial applications. Filamentous moulds in traditionally prepared starters of North East India have ethnical, socio-cultural, microbiological and functional properties.

Highlights of Main Findings

- Reports on fungal composition of traditionally prepared dry starters of North East India viz. *marcha* of Sikkim, *thiat* of Meghalaya, *humao* of Assam, *hamei* of Manipur, *chowan* of Tripura, *phut* of Arunachal Pradesh, *dawdim* of Mizoram and *khekhrii* of Nagaland.
- Fungal taxonomical keys were prepared based on macro- and micro-morphology and ITS sequence data and identified 7 genera with 16 species of filamentous moulds: *Mucor circinelloides* (20%), *Aspergillus sydowii* (11%), *Penicillium chrysogenum* (11%), *Bjerkandera adusta* (11%), *Penicillium citrinum* (7%), *Rhizopus oryzae* (7%), *Aspergillus niger* (5%), *Aspergillus flavus* (5%), *Mucor indicus* (5%) *Rhizopus microsporus* (5%), *Rhizopus delemar* (2%), *Aspergillus versicolor* (2%), *Penicillium oxalicum* (2%), *Penicillium polonicum* (2%), *Trametes hirsuta* (2%) and *Cladosporium parahalotolerans* (2%).
- *Mucor circinelloides* is dominant fungus in starter cultures of North East India.
- This is the first attempt to illustrate the taxonomical keys of filamentous fungi isolated from traditionally prepared dry starters of North East India to species level, based on morphological and molecular identifications.
- Fungal species present in dry starters are morphologically, ecologically and phylogenetically diverse.
- Fungal species showed high extracellular enzymatic and antinutritive degrading activities indicating their functionality in dry starters.

Schematic Representation of Complete PhD work



BIBILIOGRAPHY

Abdelrazek, N.A., Elkhatib, W.F., Raafat, M.M. and Aboulwafa, M. (2019). Diverse origins of microbial L-asparaginases and their current miscellaneous applications. *Archives of Pharmaceutical Sciences Ain Shams University* 3(1): 21-36.

Abubakar, A., Suberu, H.A., Bello, I.M., Abdulkadir, R., Daudu, O.A. and Lateef, A.A. (2013). Effect of pH on mycelial growth and sporulation of *Aspergillus parasiticus. Journal of Plant Sciences* 1 (4): 64-67.

Achaya, K.T. (1991). Alcoholic fermentation and its products in ancient India. *Indian Journal of History of Science* 26(2): 123-129.

Adamczyk, B., Simon, J., Kitunen, V., Adamczyk, S. and Smolander, A. (2017). Tannins and their complex interaction with different organic nitrogen compounds and enzymes: old paradigms versus recent advances. *Chemistry Open* 6(5): 610-614.

Adebo, O.A., Kayitesi, E. and Njobeh, P.B. (2019). Reduction of mycotoxins during fermentation of whole grain sorghum to whole grain ting (a Southern African Food). *Toxins* 11(3):180. doi.org/10.3390/toxins11030180.

Adekoya, I., Obadina, A., Phoku, J., Nwinyi, O. and Njobeh, P. (2017). Contamination of fermented foods in Nigeria with fungi. *LWT- Food Science and Technology* 86: 76-84.

Adeyemo, S.M. and Onilude, A.A. (2013). Enzymatic reduction of anti-nutritional factors in fermenting soybeans by *Lactobacillus plantarum* isolates from fermenting cereals. *Nigerian Food Journal* 31(2): 84-90.

Afinah, S., Yazid, A.M., Anis Shobirin, M.H. and Shuhaimi, M. (2010). Phytase: application in food industry. *International Food Research Journal* 17(1): 13-21.

Agrahar-Murugkar, D. and Subbulakshmi, G. (2006). Preparation techniques and nutritive value of fermented foods from the Khasi tribes of Meghalaya. *Ecology of Food and Nutrition* 45(1): 27-38.

Agrawal, K., Chaturvedi, V. and Verma, P. (2018). Fungal laccase discovered but yet undiscovered. *Bioresources and Bioprocessing* 5(1): 4. doi.org/10.1186/s40643-018-0190-z.

Agusti-Brisach, C. and Armengol, J. (2012). Effects of temperature, pH and water potential on mycelial growth, sporulation and chlamydospore production in culture of *Cylindrocarpon* spp. associated with black foot of grapevines. *Phytopathologia Mediterranea* 51(1): 37-50.

Ahmad, A., Anjum, A.A., Rabbani, M., Ashraf, K., Nawaz, M., Sana, S. and Asif, A. (2018). Diversity of phytase producing non-toxigenic fungi isolated from soil. *Journal of Animal and Plant Sciences* 28(2): 467-473.

Ahmed, A. and Bibi, A. (2018). Fungal cellulase; production and applications: minireview. *LIFE: International Journal of Health and Life-Sciences* 4(1): doi: 10.20319/lijhls.2018.41.1936.

Aidoo, K.E. and Nout, M.R. (2010). Functional yeasts and molds in fermented foods and beverages. In: *Fermented foods and beverages of the world* (Eds: Tamang, J.P. and Kailasapathy, K.), pp. 127-148. CRC Press, Taylor & Francis Group, New York.

Akasaka, N., Kato, S., Kato, S., Hidese, R., Wagu, Y., Sakoda, H. and Fujiwara, S. (2018). Agmatine production by *Aspergillus oryzae* is elevated by low pH during solid-state cultivation. *Applied Environmental Microbiology* 84(15): e00722-18. doi: 10.1128/AEM.00722-18.

Akatın, M.Y. (2019). An Overview of Amylase Production by Solid State Fermentation (SSF) since 2010. *Teknik Bilimler Dergisi* 9(1): 1-7.

Akula, S. and Golla, N. (2018). Optimization of cellulase production by *Aspergillus niger* isolated from forest soil. *The Open Biotechnology Journal* 12(1). doi: 10.2174/1874070701812010256.

Ali, S.R., Fradi, A.J. and Al-Aaraji, A.M. (2017). Effect of some physical factors on growth of five fungal species. *European Academic Research* (2):1069-1078

Al-Maqtari, Q.A., Waleed, A.A. and Mahdi, A.A. (2019). Microbial enzymes produced by fermentation and their applications in the food industry-A review. *International Journal of Agriculture Innovations and Research* 8(1): 2319-147.

Al-Mraai, S.T.Y., Al-Fekaiki, D.F. and Al-Manhel, A.J.A. (2019). Purification and characterization of tannase from the local isolate of *Aspergillus niger*. *Journal of Applied Biology and Biotechnology* 7(01): 029-034.

Alsohaili, S.A. and Bani-Hasan, B.A. (2018). Morphological and molecular identification of fungi isolated from different environmental sources in the Northern Eastern desert of Jordan. *Jordan Journal of Biological Sciences* 11: 329-337.

Alves, M.H., Campos-Takaki, G.M., Porto, A.L.F. and Milanez, A.I. (2002). Screening of *Mucor* spp. for the production of amylase, lipase, polygalacturonase and protease. *Brazilian Journal of Microbiology* 33(4): 325-330.

Anal, A.K. (2019). Quality ingredients and safety concerns for traditional fermented foods and beverages from Asia: A review. *Fermentation* 5(1): 8. doi.org/10.3390/fermentation5010008.

Andersen, B., Frisvad, J.C., Søndergaard, I., Rasmussen, I.S. and Larsen, L.S. (2011). Associations between fungal species and water-damaged building materials. *Applied and Environmental Microbiology* 77(12): 4180–4188.

Andrew, W. (1992). Manual of food quality control. Food and Agriculture Organization of the United Nations.

Angmo, K. and Bhalla, T.C. (2014). Preparation of Phabs-an indigenous starter culture for production of traditional alcoholic beverage, Chhang, in Ladakh. *Indian Journal of Traditional Knowledge* 13(2): 347-351.

Anupma, A., Pradhan, P., Sha, S.P. and Tamang, J.P. (2018). Traditional skill of ethnic people of the Eastern Himalayas and North East India in preserving microbiota as dry amylolytic starters. *Indian Journal of Traditional Knowledge* 17: 184-190.

Ansorena, D. and I. Astiasar an. 2016. Fermented foods: Composition and health effects. In *Encyclopaedia of food and health*'s (Eds: Cabellero, B., Finglas, P.M. and Toldra, F.), pp. 649–55. Oxford: Academic Press, UK.

Arregui, L., Ayala, M., Gómez-Gil, X., Gutiérrez-Soto, G., Hernández-Luna, C.E., de los Santos, M.H. and Trujillo-Roldán, M.A. (2019). Laccases: structure, function, and potential application in water bioremediation. *Microbial Cell Factories* 18(1): 200. doi.org/10.1186/s12934-019-1248-0.

Asres, D.T., Nana, A. and Nega, G. (2018). Complementary feeding and effect of spontaneous fermentation on anti-nutritional factors of selected cereal-based complementary foods. *BMC Pediatrics* 18(1): 394. doi.org/10.1186/s12887-018-1369-3.

Ayinla, Z.A., Ademakinwa, A.N. and Agboola, F.K. (2017). Studies on the optimization of lipase production by *Rhizopus* sp. zac3 isolated from the contaminated soil of a palm oil processing shed. *Journal of Applied Biology & Biotechnology* 5(02): 030-037.

Bachmann, H., Pronk, J.T., Kleerebezem, M. and Teusink, B. (2015). Evolutionary engineering to enhance starter culture performance in food fermentations. *Current Opinion in Biotechnology* 32: 1-7.

Bae, H.D., Yanke, L.J., Cheng, K.J. and Selinger, L.B. (1999). A novel staining method for detecting phytase activity. *Journal of Microbiological Methods* 39(1): 17-22.

Bahiru, B., Mehari, T. and Ashenafi, M. (2006). Yeast and lactic acid flora of tej, an indigenous Ethiopian honey wine: variations within and between production units. *Food Microbiology* 23(3): 277-282.

Bailey, M.J., Biely, P. and Poutanen, K. (1992). Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology* 23(3): 257-270.

Bajaj, I., Veiga, T., van Dissel, D., Pronk, J.T. and Daran, J.M. (2014). Functional characterization of a *Penicillium chrysogenum* mutanase gene induced upon co-cultivation with *Bacillus subtilis*. *BMC Microbiology* 14: 114. doi: 10.1186/1471-2180-14-114.

Bal, J., Yun, S.H., Yeo, S.H., Kim, J.M. and Kim, D.H. (2016). Metagenomic analysis of fungal diversity in Korean traditional wheat-based fermentation starter nuruk. *Food Microbiology* 60: 73-83.

Banerjee, D. and Mahapatra, S. (2012). Fungal tannase: a journey from strain isolation to enzyme applications. *Dynamic Biochemistry, Process Biotechnology and Molecular Biology* 6(2): 49-60.

Banerjee, G. and Ray, A.K. (2017). Impact of microbial proteases on biotechnological industries. *Biotechnology and Genetic Engineering Reviews* 33(2): 119-143.

Barooah, M., Bora, S.S. and Goswami, G. (2020). Ethnic fermented foods and beverages of Assam. In: *Ethnic Fermented Foods and Alcoholic Beverages of India: Science History and Culture* (Ed: Tamang, J.P.), pp. 85-104. Springer Nature, Singapore.

Bavaro, S.L., Susca, A., Frisvad, J.C., Tufariello, M., Chytiri, A., Perrone, G., Mita, G., Logrieco. A.F. and Bleve, G. (2017). Isolation, characterization, and selection of molds associated to fermented black table olives. *Frontiers in Microbiology* 8: 1356. doi:10.3389/fmicb.2017.01356.

Bedaiwy, M.Y., Awadalla, O.A., Abou-Zeid, A.M. and Hamada, H.T. (2016). Optimal conditions for production of L-asparaginase from *Aspergillus tamarii*. *Egyptian Journal of Experimental Biology (Botany)* 12(2): 229-237.

Belda, I., Ruiz, J., Esteban-Fernández, A., Navascués, E., Marquina, D., Santos, A. and Moreno-Arribas, M.V. (2017). Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules* 22: 189. doi:10.3390/molecules22020189.

Beniwal, V., Kumar, A., Sharma, J. and Chhokar, V. (2013). Recent advances in industrial application of tannases: a review. *Recent Patents on Biotechnology* 7(3): 228-233.

Bensch. K., Braun, U., Groenewald, J.Z. and Crous, P.W. (2012). The genus Cladosporium. *Studies in Mycology* 72: 1-401.

Bhardwaj, K., Raju, A. and Rajasekharan, R. (2001). "Identification, purification and characterization of a thermally stable lipase from rice bran. A new member of the phosphoslipase family". *Plant Physiology* 127: 1728–38.

Bhardwaj, K.N., Jain, K.K., Kumar, S. and Kuhad, R.C. (2016). Microbiological Analyses of Traditional Alcoholic Beverage (Chhang) and its Starter (Balma) Prepared by Bhotiya Tribe of Uttarakhand, India. *Indian Journal of Microbiology* 56(1): 28-34.

Bhardwaj, N., Kumar, B. and Verma, P. (2019). A detailed overview of xylanases: an emerging biomolecule for current and future prospective. *Bioresources and Bioprocessing* 6(1): 40. doi.org/10.1186/s40643-019-0276-2.

Bhatt, K.C., Malav, P.K. and Ahlawat, S.P. (2018). 'Jumin'a traditional beverage of Nocte tribe in Arunachal Pradesh: an ethnobotanical survey. *Genetic Resources and Crop Evolution* 65(2): 671-677.

Bhavsar, K., Buddhiwant, P., Soni, S.K., Depan, D., Sarkar, S. and Khire, J.M. (2013). Phytase isozymes from Aspergillus niger NCIM 563 under solid state fermentation: Biochemical characterization and their correlation with submerged phytases. *Process Biochemistry* 48(11): 1618-1625.

Blaalid, R., Kumar, S., Nilsson, R.H., Abarenkov, K., Kirk, P.M. and Kauserud, H. (2013). ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources*13(2): 218-224.

Blackwell, M. (2011). The Fungi: 1, 2, 3… 5.1 million species?. *American Journal of Botany* 98(3): 426-438.

Blandino, A., Al-Aseeri, M.E., Pandiella, S.S., Cantero, D. and Webb, C. (2003). Cereal-based fermented foods and beverages. *Food Research International* 36: 527–543.

Bokulich, N.A., Lucy Joseph, C.M., Allen, G., Benson, A.K. and Mills, D.A. (2012). Next-generation sequencing reveals significant bacterial diversity of Botrytized wine. *PLoS ONE* 7: e36357 doi: 10.1371/journal.pone.0036357.

Bokulich, N.A., Ohta, M., Lee, M. and Mills, D.A. (2014). Indigenous bacteria and fungi drive traditional kimoto sake fermentations. *Applied and Environmental Microbiology* 80(17): 5522-5529.

Bora, S.S., Keot, J., Das, S., Sarma, K. and Barooah, M. (2016). Metagenomics analysis of microbial communities associated with a traditional rice wine starter culture (Xaj-pitha) of Assam, India. *3 Biotech* 6(2):153. doi: 10.1007/s13205-016-0471-1.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P. and Powell, I.B. (2012). Food fermentations: microorganisms with technological beneficial use. *International Journal of Food Microbiology* 154(3): 87-97.

Bradoo, S., Gupta, R. and Saxena, R.K. (1996). Screening of extracellular tannase-producing fungi: Development of a rapid and simple plate assay. *The Journal of General and Applied Microbiology* 42(4): 325-329.

Brahmbhatt, D., Modi, H.A. and Jain, N.K. (2014). Preliminary isolation and screening of tannase producing bacteria and fungi. *International Journal of Current Microbiology and Applied Sciences* 3(11): 193-203.

Brandt, M.J. (2014). Starter cultures for cereal based foods. *Food Microbiology* 37: 41-43.

Burge, H. (2006). How does heat affect fungi. *The Environmental Reporter* 4(3): 1-3.

Caballero, B., Trugo, L.C. and Finglas, P.M. (2003). Encyclopedia of food sciences and nutrition. Academic.

Cachumba, J.J.M., Antunes, F.A.F., Peres, G.F.D., Brumano, L.P., Dos Santos, J.C. and Da Silva, S.S. (2016). Current applications and different approaches for microbial l-asparaginase production. *Brazilian Journal of Microbiology* 47: 77-85.

Cairns, T.C., Nai, C. and Meyer, V. (2018). How a fungus shapes biotechnology: 100 years of *Aspergillus niger* research. *Fungal Biology and Biotechnology* 5(13) doi.org/10.1186/s40694-018-0054-5.

Calle-Vallejo, F. and Koper, M. (2013). Theoretical considerations on the electroreduction of CO to C_2 species on Cu (100) electrodes. *Angewandte Chemie* 125(28): 7423-7426.

Calmette, A. (1892). Contribution à l´étude des ferments de l´amidon. La levure chinoise. *Annales de IInstitut. Pasteur* 6: 604-622.

Campbell-Platt, G. (1987). *Fermented foods of the world: A dictionary and guide*. London: Butterworths Scientific.

Caplice, E. and Fitzgerald, G.F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology* 50(1-2): 131-149.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. and Huttley, G.A. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5): 335. doi: 10.1038/nmeth.f.303.

Carlile, M.J., Watkinson, S.C. and Gooday, G.W. (2001) The Fungi. London, UK: Academic Press.

Carrasco, M., Villarreal, P., Barahona, P., Alcaíno, J., Cifuentes, V. and Baeza, M. (2016). Screening and characterization of amylase and cellulase activities in psychrotolerant yeasts. *BMC Microbiology* 16:21.

Carrière, F., Thirstrup, K., Hjorth, S. and Boel, E. (1994). Cloning of the classical guinea pig pancreatic lipase and comparison with the lipase related protein 2. *FEBS Letters* 338(1): 63-68.

Carroll, E., Trinh, T.N., Son, H., Lee, Y.W. and Seo, J.A. (2017). Comprehensive analysis of fungal diversity and enzyme activity in nuruk, a Korean fermenting starter, for acquiring useful fungi. *Journal of Microbiology* 55: 357. doi: 10.1007/s12275-017-7114-z.

Cavalcanti, R.M.F., Ornela, P.H.O., Jorge, J.A. and Guimaraes, L.H.S. (2017). Screening, Selection and optimization of the culture conditions for tannase production by endophytic fungi isolated from Caatinga. *Journal of Applied Biology and Biotechnology* 5:1-9.

Chakrabarty, J., Sharma, G.D. and Tamang, J.P. (2014). Traditional technology and product characterization of some lesser-known ethnic fermented foods and beverages of North Cachar Hills District of Assam. *Indian Journal of Traditional Knowledge* 13 (4): 706-715.

Chay, C., Dizon, E.I., Elegado, F.B., Norng, C., Hurtada, W.A. and Raymundo, L.C. (2017). Isolation and identification of molds and yeasts in medombae, a rice wine starter culture from Kompong Cham Province, Cambodia. *Food Research* 1(6):213-220.

Chen, B., Wu, Q. and Xu, Y. (2014). Filamentous fungal diversity and community structure associated with the solid-state fermentation of Chinese Maotai-flavor liquor. *International Journal of Food Microbiology* 179: 80-84.

Chettri, N., Sharma, E., Shakya, B., Thapa, R., Bajracharya, B., Uddin, K., Oli, K.P. and Choudhury, D. (2010). Biodiversity in the Eastern Himalayas: status, trends and vulnerability to climate change. Technical Report 2, ICIMOD, Kathmandu.

Chim, C., Erlinda, I.D., Elegado, F.B., Hurtada, A.W., Chakrya, N. and Raymundo, C.L. (2015). Traditional dried starter culture (Medombae) for rice liquor production in Cambodia. *International Food Research Journal* 22(4): 1642.

Cho, H.K., Seo, W.T., Lee, J.Y. and Cho, K.M. (2012). Quality characteristics of cereal makgeolli rice nuruk prepared *Rhizopus oryzae* CCS01. *Journal of the Korean Society of Food Science and Nutrition* 41(7): 1002-1008.

Choi, Y.W., Hodgkiss, I.J. and Hyde, K.D. (2005). Enzyme production by endophytes of *Brucea javanica. International Journal of Agricultural Technology* 55-66.

Choudhary, V. and Jain, P.C. (2012). Screening of alkaline protease production by fungal isolates from different habitats of Sagar and Jabalpur district (MP). *Journal of Academic and Industrial Research* 1(4): 215-220.

Chuzho, K., Dkhar, M.S. and Lyngdoh, A. (2017). Wood-rotting fungi in two forest stands of Kohima, North-East India: A preliminary report. *Current Research in Environmental & Applied Mycology*. 7(1): 1-7.DOI: 10.5943/cream/7/1/1.

Cilerdzic, J., Stajic, M., Vukojevic, J., Duletic-Lausevic, S. and Knezevic, A. (2011). Potential of *Trametes hirsuta* to produce ligninolytic enzymes during degradation of agricultural residues. *Bioresources* 6(3): 2885-2895.

Colen, G., Junqueira, R.G. and Moraes-Santos, T. (2006). Isolation and screening of alkaline lipase-producing fungi from Brazilian savanna soil. *World Journal of Microbiology and Biotechnology* 22(8): 881-885.

Collins, T., Gerday, C. and Feller, G. (2005). Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiology Reviews* 29(1): 3-23.

Costello, M.J., May, R.M. and Stork, N.E. (2013). Can we name Earth‘s species before they go extinct? *Science* 339: 413–416.

Couto, S.R., Rosales, E. and Sanromán, M.A. (2006). Decolourization of synthetic dyes by *Trametes hirsuta* in expanded-bed reactors. *Chemosphere* 62(9): 1558-1563.

Cunha, L., Martarello, R., Souza, P.M.D., Freitas, M.M.D., Barros, K.V.G., Ferreira Filho, E.X., Mello, M.H. and Magalhães, P.O. (2018). Optimization of xylanase production from *Aspergillus foetidus* in soybean residue. *Enzyme Research* 2018. doi.org/10.1155/2018/6597017.

Da Cunha, M.C., dos Santos Aguilar, J.G., de Melo, R.R., Nagamatsu, S.T., Ali, F., de Castro, R.J.S. and Sato, H.H. (2019). Fungal L-asparaginase: Strategies for production and food applications. *Food Research International* 108658. Doi.10.1016/j.foodres.2019.108658.

Das, C.P. and Pandey, A. (2007). Fermentation of traditional beverages prepared by Bhotiya community of Uttaranchal Himalaya. *Indian Journal of Traditional Knowledge* 6(1): 136-140.

Das, A.J. and Deka, S.C. (2012). Fermented foods and beverages of the North-East India. *International Food Research Journal* 19(2): 377-392.

Das, A.J., Deka, S.C. and Miyaji, T. (2012a). Methodology of rice beer preparation and various plant materials used in starter culture preparation by some tribal communities of North-East India: A survey. *International Food Research Journal* 19(1): 101.

Das, A.J., Miyaji, T. and Deka, S.C. (2017). Amylolytic fungi in starter cakes for rice beer production. *The Journal of General and Applied Microbiology* 63(4): 236-245.

Daroonpunt, R., Tanasupawat, S. and Keeratipibul, S. (2016). Characterization and amylolytic activity of yeast and mold strains from Thai sweet rice. *Malaysian Journal of Microbiology* 12(2): 121-131.

Debnath, G., Das, P. and Saha, A.K. (2020). Screening and characterization of extracellular cellulase enzyme produced by wild edible mushroom *Pleurotus giganteus*. *Indian Journal of Natural Products and Resources* 10(3): 195-199.

Dellamora-Ortiz, G.M., Martins, R.C., Rocha, W.L. and Dias, A.P. (1997). “Activity and stability of a *Rhizomucor miehei* lipase in hydrophobic media”. *Biotechnology and Applied Biochemistry* 26: 31-37.

Dennis, C. and Blijham, J.M. (1980). Effect of temperature on viability of sporangiospores of *Rhizopus* and *Mucor* species. *Transactions of the British Mycological Society* 74(1): 89-94.

Deori, C., Begum, S.S. and Mao, A.A. (2007). Ethnobotany of Sujen-A local rice beer of Deori tribe of Assam. *Indian Journal of Traditional Knowledge* 6(1): 121-125.

Dhakar, K. and Pandey, A. (2013). Laccase production from a temperature and pH tolerant fungal strain of Trametes hirsuta (MTCC 11397). *Enzyme Research* 2013. doi.org/10.1155/2013/869062.

Dipak, V. and Sheela, S. (2015) Isolation of tannase producers from soil. *International Journal of Research in Biosciences* 4(3): 49-55.

Doi, S.A., Pinto, A.B., Canali, M.C., Polezel, D.R., Chinellato, R.A.M. and Oliveira, A.J.F.C. (2018). Density and diversity of filamentous fungi in the water and sediment of Araçá bay in São Sebastião, São Paulo, Brazil. *Biota Neotropica Campinas* 18(1): e20170416. doi.org/10.1590/1676-0611-bn-2017-0416.

Dolci, P., Alessandria, V., Rantsiou, K. and Cocolin, L. (2015). "Advanced methods for the identification, enumeration, and characterization of microorganisms in fermented foods". *In: Advances in Fermented Foods and Beverages* (Ed: Holzapfel, W.H.), pp. 157-176. Elsevier, London. doi:10.1016/b978-1-78242-015-6.00007-4.

Doriya, K. and Kumar, D.S. (2016). Isolation and screening of L-asparaginase free of glutaminase and urease from fungal sp. *3 Biotech* 6(2): 239. doi: 10.1007/s13205-016-0544-1.

Drozłowska, E. (2019). The use of enzymatic fungal activity in the food industry-review. *World Scientific News* 116: 222-229.

Dunbar, R.I.M., Launay, J., Wlodarski, R., Robertson, C., Pearce, E., Carney, J. and MacCarron, P. (2017). Functional benefits of (modest) alcohol consumption. *Adaptive Human Behavior and Physiology* 3: 118–133.

Dung, N.N.T.P. (2004). Defined fungal starter granules for purple glutinous rice wine. Ph.d. Thesis. Wageningen University, Wageningen, The Netherlands.pp110.

Dung, N.T.P., Rombouts, F.M. and Nout, M.J.R. (2005). Development of defined mixed-culture fungal fermentation starter granulate for controlled production of rice wine. *Innovative Food Science and Emerging Technologies* 6(4): 429-441.

Dung, N.T.P., Rombouts, F.M. and Nout, M.J.R. (2006). Functionality of selected strains of moulds and yeasts from Vietnamese rice wine starters. *Food Microbiology* 23(4): 331-340.

Dung, N.T.P., Rombouts, F.M. and Nout, M.J.R. (2007). Characteristics of some traditional Vietnamese starch-based rice wine fermentation starters (men). *Food Science and Technology Lebensmittel-Wissenschaft* and *Technologie* 40(1): 130-135.

Dupont, J., Dequin, S., Giraud, T., Le Tacon, F., Marsit, S., Ropars, J., Franck Richard, F. and Selosse, M.A. (2017). Fungi as a source of food. In: *The Fungal Kingdom*

(Eds: Heitman, J., Howlett, B.J., Crous, P.W., Stukenbrock, E.H., James, T.Y. and Gow, N.A.R.), pp. 1063-1085. American Society for Microbiology, USA.

Eddouzi, J., Lohberger, A., Vogne, C., Manai, M. and Sanglard, D. (2013). Identification and antifungal susceptibility of a large collection of yeast strains isolated in Tunisian hospitals. *Medical Mycology* 51(7): 737-746.

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26: 2460–2461.

Elad, Y. and Kapat, A. (1999). The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. *European Journal of Plant Pathology* 105(2): 177-189.

Elegado, F.B., Colegio, S.M.T., Lim, V.M.T., Gervasio, A.T.R., Perez, M.T.M., Balolong, M.P. and Mendoza, B.C. (2016). Ethnic fermented foods of the philippines with reference to lactic acid bacteria and yeasts. In: *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (Ed: Tamang, J.P.), pp. 323-340. Springer, New Delhi.

El-Batal, A.I. and Karem, H.A. (2001). Phytase production and phytic acid reduction in rapeseed meal by *Aspergillus niger* during solid state fermentation. *Food Research International* 34(8): 715-720.

El-Fallal, A., Dobara, M.A., El-Sayed, A. and Omar, N. (2012). Starch and microbial α-amylases: from concepts to biotechnological applications. In *Carbohydrates–Comprehensive Studies on Glycobiology and Glycotechnology* (Ed: Chang, C.F.), pp. 459-488. InTech Croatia.

El-Ghonemy, D.H., El-Gamal, M.S., Tantawy, A.E. and Ali, T.H. (2017). Extracellular alkaline lipase from a novel fungus *Curvularia* sp. DHE 5: Optimisation of physicochemical parameters, partial purification and characterisation. *Food Technology and Biotechnology* 55(2): 206-217.

Ezekiel, C.N., Ayeni, K.I., Misihairabgwi, J.M., Somorin, Y.M., Chibuzor-Onyema, I.E., Oyedele, O.A., Abia, W.A., Sulyok, M., Shephard, G.S. and Krska, R. (2018). Traditionally processed beverages in Africa: a review of the mycotoxin occurrence patterns and exposure assessment. *Comprehensive Reviews in Food Science and Food Safety* 17: 334-351.

Fadahunsi, I.F. and Garuba, O.E. (2012). Amylase production by *Aspergillus flavus* associated with the bio-deterioration of starch-based fermented foods. *New York Science Journal* 5(1): 13-18.

Farias, N., Almeida, I. and Meneses, C. (2018). New bacterial phytase through metagenomic prospection. *Molecules* 23(2): 448. doi.10.3390/molecules23020448.

Fentahun, M. and Kumari, P.V. (2017). Isolation and screening of amylase producing thermophilic spore forming Bacilli from starch rich soil and characterization of their amylase activity. *African Journal of Microbiology Research* 11(21): 851-859.

Fernandes, E.G., Pereira, O.L., da Silva, C.C., Bento, C.B.P. and de Queiroz, M.V. (2015). Diversity of endophytic fungi in *Glycine max*. *Microbiological Research* 181: 84-92.

Figueiredo, R., Araújo, P., Llerena, J.P.P. and Mazzafera, P. (2019). Suberin and hemicellulose in sugarcane cell wall architecture and crop digestibility: A biotechnological perspective. *Food and Energy Security* 8(3): e00163. doi.org/10.1002/fes3.163.

Fouda, A.H., Hassan, S.E.D., Eid, A.M. and Ewais, E.E.D. (2015). Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). *Annals of Agricultural Sciences* 60(1): 95-104.

Frisvad, J.C., Møller, L.L., Larsen, T.O., Kumar, R. and Arnau, J. (2018). Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger, Aspergillus oryzae,* and *Trichoderma reesei. Applied Microbiology and Biotechnology* 102(22): 9481-9515.

Fronteras, J.P. and Bullo, L.L.R. (2017). Raw starch-digesting amylase from *Saccharomycopsis fibuligera* 2074 isolated from bubod starter. *Philippine Journal of Science* 146 (1): 27-35.

Furini, G., Berger, J.S., Campos, J.A. Sand, S.T. and Germani, J.C. (2018). Production of lipolytic enzymes by bacteria isolated from biological effluent treatment systems. *Anais da Academia Brasileira de Ciências* 90(3): 2955-2965.

Gaddeyya, G., Niharika, P.S., Bharathi, P. and Kumar, P.R. (2012). Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. *Advances in Applied Science Research* 3(4): 2020-2026.

Gan, S.H., Yang, F., Sahu, S. K., Luo, R.Y., Liao, S.L., Wang, H.Y., Jin, T., Wang, L., Zhang, P.F., Liu, X., Xu, J., Xu, J., Wang, Y.Y. and Liu, H. (2019). Deciphering

the composition and functional profile of the microbial communities in Chinese moutai liquor starters. *Frontiers in Microbiology* 10: 1540. doi.org/10.3389/fmicb.2019.01540.

Gargova, S., Roshkova, Z. and Vancheva, G. (1997). Screening of fungi for phytase production. *Biotechnology Techniques* 11(4): 221-224.

Geetha, K.N., Jeyaprakash, K. and Nagaraja, Y.P. (2011). Isolation, screening of *Aspergillus flavus* and its production parameters for α-amylase under solid state fermentation. *Journal of Applied and Natural Science* 3(2): 268-273.

Geisen, R. (1993). Fungal starter cultures for fermented foods: molecular aspects. *Trends in Food Science and Technology* 4(8): 251-256.

Geoffry, K. and Achur, R.N. (2018). Screening and production of lipase from fungal organisms. *Biocatalysis and Agricultural Biotechnology* 14: 241-253

Ghosh, B. and Ray, R.R. (2011). Current commercial perspective of *Rhizopus oryzae*: a review. *Journal of Applied Sciences* 11 (14): 2470–2486.

Ghosh, K., Ray, M., Adak, A., Dey, P., Halder, S.K., Das, A., Jana, A., Parua, S., Das, Mohapatra, P.K., Pati, B.R. and Mondal, K.C. (2015). Microbial, saccharifying and antioxidant properties of an Indian rice based fermented beverage. *Food Chemistry* 168: 196-202.

Ghosh, S., Rahaman, L., Kaipeng, D.L., Deb, D., Nath, N., Tribedi, P. and Sharma, B.K. (2016). Community-wise evaluation of rice beer prepared by some ethnic tribes of Tripura. *Journal of Ethnic Foods* 3(4): 251-256.

Gille, D., Schmid, A., Walther, B. and Vergères, G. (2018). Fermented food and non-communicable chronic diseases: a review. *Nutrients* 10(4): 448. doi: 10.3390/nu10040448.

Giraffa, G. (2004). Studying the dynamics of microbial populations during food fermentation. *FEMS Microbiology Reviews* 28(2): 251-260.

Girdhari, S.N. and Peshwe, S.A. (2015). Isolation and Screening of Tannase producing fungi. *International Journal of Current Microbiology and. Applied Science* 4(7): 765-774.

Glazunova, O.A., Shakhova, N.V., Psurtseva, N.V., Moiseenko, K.V., Kleimenov, S.Y. and Fedorova, T.V. (2018). White-rot basidiomycetes *Junghuhnia nitida* and *Steccherinum bourdotii:* Oxidative potential and laccase properties in comparison

with *Trametes hirsuta* and *Coriolopsis caperata*. *PloS One* 13(6). doi.org/10.1371/journal.pone.0197667.

Godoy, M.G., Amorim, G.M., Barreto, M.S. and Freire, D.M. (2018). Agricultural Residues as Animal Feed: Protein Enrichment and Detoxification Using Solid-State Fermentation. *In Current Developments in Biotechnology and Bioengineering* (Ed: Larroche, C., Sanroman, M., Du, G. and Pandey, A.), pp. 235-256. Elsevier.

Gopinath S.C., Anbu, P. Lakshmipriya, T. and Hilda, A. (2013). Strategies to characterize fungal lipases for applications in medicine and dairy industry. *BioMed Research International* 2013. doi.org/10.1155/2013/154549.

Gopinath, S.C., Anbu, P., Arshad, M.K., Lakshmipriya, T., Voon, C.H., Hashim, U. and Chinni, S.V. (2017). Biotechnological processes in microbial amylase production. *BioMed Research International* 2017: 9. doi.org/10.1155/2017/1272193.

Gordon, R.E., Haynes, W.C. and Pang, C.H.N. (1973). The genus bacillus. Agricultural Research Service, United States Department of Agriculture, Washington, D.C.

Goulart, A.J., Carmona, E.C. and Monti, R. (2005) Partial purification and properties of cellulase-free alkaline xylanase produced by *Rhizopus stolonifer* in solid-state fermentation. *Brazilian Archives of Biology and Technology* 48:327-33.

Greeshma, A.G., Srivastava, B. and Srivastava, K. (2006). Plants used as antimicrobials in the preparation of traditional starter cultures of fermentation by certain tribes of Arunachal Pradesh. *Bulletin of Arunachal Forest Research* 22(1&2): 52-7.

Greiner, R. and Carlsson, N.G. (2006). Myo-Inositol phosphate isomers generated by the action of a phytate-degrading enzyme from *Klebsiella terrigena* on phytate. *Canadian Journal of Microbiology* 52(8): 759-768.

Greiner, R. and Konietzny, U. (2006a). Phytase for food application. *Food Technology & Biotechnology* 44(2): 125-140.

Greppi, A., Rantsiou, K., Padonou, W., Hounhouigan, J., Jespersen, L., Jakobsen, M. and Cocolin, L. (2013). Determination of yeast diversity in ogi, mawè, gowé and tchoukoutou by using culture-dependent and-independent methods. *International Journal of Food Microbiology* 165(2): 84-88.

Griebeler, N., Polloni, A.E., Remonatto, D., Arbter, F., Vardanega, R., Cechet, J.L., Luccio, M.D., Oliveira, D.D., Treichel, H., Cansian, R.L., Rigo, E. and Ninow, J.L.

(2011). Isolation and screening of lipase-producing fungi with hydrolytic activity. *Food and Bioprocess Technology* 4(4): 578-586.

Grover, A., Arora, M. and Sarao, L.K. (2013). Production of fungal amylase and cellulase enzymes via solid state fermentation using *Aspergillus oryzae* and *Trichoderma reesei*. *International Journal of Advancements in Research & Technology* 2(8):108-124.

Gryganskyi, A.P., Golan, J., Dolatabadi, S., Mondo, S., Robb, S., Idnurm, A., Muszewska, A., Steczkiewicz, K., Masonjones, S., Liao, H.L., Gajdeczka, M.T., Anike, F., Vuek, A., Anishchenko, I.M., Voigt, K., de, Hoog, G.S., Smith, M.E., Heitman, J., Vilgalys, R. and Stajich, J.E. (2018). Phylogenetic and phylogenomic definition of *Rhizopus* species. *G3 (Bethesda)* 8(6): 2007-2018.

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K. and Chauhan, B. (2003). Microbial α-amylases: a biotechnological perspective. *Process biochemistry* 38(11): 1599-1616.

Gupta, N. (2018). Ethnicity in north-east India: A challenge to identity. *International Journal of Advanced Educational Research* 3(2): 292-294.

Gupta, P.K., Raghunath, S.S., Prasanna, D.V., Venkat, P., Shree, V., Chithananthan, C., Choudhary, K., Surender, K. and Geetha, K. (2019). An Update on Overview of Cellulose, Its Structure and Applications. *In Cellulose* (Ed. Pascual, A.R. and Martín, M.E.E). Intech Open. DOI: 10.5772/intechopen.84727.

Haard, N.F., Odunfa, S.A., Lee, C.H., Quintero-Ramírez, R., Lorence-Quiñones, A. and Wacher-Radarte, C. (1999). *Fermented Cereals: A Global Perspective. FAO Agricultural Service Bulletin* 138, pp. 63–97. Rome, Italy: Food and Agriculture Organization.

Hameed, A., Hussain, S.A., Yang, J., Ijaz, M.U., Liu, Q., Suleria, H.A.R. and Song, Y. (2017). Antioxidants potential of the filamentous fungi (*Mucor circinelloides*). *Nutrients* 9: 1101. doi: 10.3390/nu9101101.

Hammer, Ø., Harper, D.A. and Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9. http://folk.uio.no/ohammer/past.

Hanlin, R.T. (1990). Illustrated Genera of Ascomycetes. APS Press. The University of Michigan.

Hartanti, A.T., Rahayu, G. and Hidayat, I. (2015). *Rhizopus* species from fresh tempeh collected from several regions in Indonesia. *Hayati Journal of Bioscience* 22(3): 136-142.

Hawksworth, D.L. and Luecking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology Spectrum* 5(4). doi: 10.1128/microbiolspec.FUNK-0052-2016.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C. and Pegler, D.N. (1995). Ainsworth & Bisby's Dictionary of the fungi. 8th ed. Egham, United Kingdom: International Mycological Institute.

He, G., Huang, J., Zhou, R., Wu, C. and Jin, Y. (2019). Effect of fortified daqu on the microbial community and flavor in Chinese strong-flavor liquor brewing process. *Frontiers in Microbiology* 10: 56. doi.org/10.3389/fmicb.2019.00056.

Herrera Bravo de Laguna, I., Toledo Marante, F.J. and Mioso, R. (2015). Enzymes and bioproducts produced by the ascomycete fungus *Paecilomyces variotii*. *Journal of Applied Microbiology* 119(6): 1455-1466.

Hesseltine, C.W. (1991). Zygomycetes in food fermentations. *Mycologist* 5(4): 162-169.

Hesseltine, C.W. and Kurtzman, C.P. (1990). Yeasts in amylolytic food starters. *Anales del Instituo Biologia University Nacional Autónoma de México. Serie Botanica.* 1–7.

Hesseltine, C.W. and Ray, M.L. (1988). Lactic acid bacteria in *murcha* and *ragi*. *Journal of Applied Microbiology* 64 (5): 395-401.

Hesseltine, C.W., Rogers, R. and Winarno, F.G. (1988). Microbiological studies on amylolytic Oriental fermentation starters. *Mycopathologia* 101: 141-155.

Hibbett, D.S., Ohman, A., Glotzer, D., Nuhn, M., Kirk, P. and Nilsson, R.H. (2011). Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. *Fungal Biology Reviews* 25(1): 38-47.

Hibbett, D., Abarenkov, K., Kõljalg, U., Öpik, M., Chai, B., Cole, J., Wang, Q., Crous, P., Robert, V., Helgason, T., Herr, J.R., Kirk, P., Lueschow, S., O'Donnell, K., Nilsson, R.H., Oono, R., Schoch, C., Smyth, C., Walker, D.M., Porras-Alfaro, A., Taylor, J.W. and Geiser, D.M. (2016). Sequence-based classification and identification of fungi *Mycologia* 108(6): 1049-1068.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk,P.M., Luckin, R., Lumbsch, H.T., Lutzoni, F., Matheny, P.B., Mclaughlin, D.J., Powell, M.J., Redhead, S., Schoch,C.L., Spatafora, J.W., Stalpers, J.A.,Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Ljalg, U.K., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowsk, J., Miller, A., Moncalvo, J.M., Standridge, S.M., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schußler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J. and Zhang, N. (2007). A higher-level phylogenetic classification of the fungi. *Mycological Research* 111(5): 509-547.

Hofrichter, M. (2010). Mycota X: Industrial Applications (Vol. 10). Springer Science & Business Media.

Hofstetter, V., Buyck, B., Eyssartier, G., Schnee, S. and Gindro, K. (2019). The unbearable lightness of sequenced-based identification. *Fungal Diversity* 96: 243–284.

Holzapfel, W.H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*, 75(3): 197-212.

Holzapfel, W. (1997). Use of starter cultures in fermentation on a household scale. *Food Ccontrol* 8(5-6): 241-258.

Horisawa, S., Inoue, A. and Yamanaka, Y. (2019). Direct ethanol production from lignocellulosic materials by mixed culture of wood rot fungi *Schizophyllum commune*, *Bjerkanderaadusta*, and *Fomitopsis palustris*. *Fermentation*. 5(1): 21. doi.org/10.3390/fermentation5010021.

Hossain, M.T., Das, F.L.O.R.A., Marzan, L.W., Rahman, M.S. and Anwar, M.N. (2006). Some properties of protease of the fungal strain *Aspergillus flavus*. *International Journal of Agriculture and Biology* 8(2): 162-164.

Howson, S.J. and Davis, R.P. (1983). Production of phytate-hydrolysing enzyme by some fungi. *Enzyme and Microbial Technology* 5(5): 377-382.

Huang, Y., Busk, P.K., Grell, M.N., Zhao, H. and Lange, L. (2014). Identification of a β-glucosidase from the *Mucor circinelloides* genome by peptide pattern recognition. *Enzyme and Microbial Technology* 67: 47-52.

Huang, Y., Yi, Z., Jin, Y., Zhao, Y., He, K., Liu, D., Zhao, D., Luo, H., Zhang, W., Fang, Y. and Zhao, H. (2017). New microbial resource: microbial diversity, function and dynamics in Chinese liquor starter. *Scientific Reports* 7(1):1-14.

Hui, Y.H. and Sherkat, F. (2005). Handbook of food science, technology, and engineering (Vol. 4), pp: 3632. Boca Raton, Fla.: CRC press.

Hui, Y.H., Evranuz, E.Ö., Arroyo-López, F.N., Fan, L., Hansen, Å.S., Jaramillo-Flores, M.E., Rakin, M., Schwan, R.F. and Zhou, W. (2012). Handbook of plant-based fermented food and beverage technology. Edition 2[nd](Vol. 134). Boca Raton, Fla: CRC Press.

Hunt, C.J., Tanksale, A. and Haritos, V.S. (2016). Biochemical characterization of a halotolerant feruloyl esterase from *Actinomyces* spp.: refolding and activity following thermal deactivation. *Applied Microbiology and Biotechnology* 100(4): 1777-1787.

Hyde, K.D., Xu, J., Rapior, S., Jeewon, R., SLumyong, S., Niego, A.G.T., Abeywickrama, P.D., Aluthmuhandiram, J.V.S., Brahamanage, R.S., Brooks, S., Chaiyasen, A., Chethana, K.W.T., Chomnunti, P., Chepkirui, C., Chuankid, B. and Silva, N.I. (2019). The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity* 97: 1–136.

Ikram-Ul-haq, H.M. and Umber, H. (2006). Production of protease by *Penicillium chrysogenum* through optimization of environmental conditions. *Journal of Agriculture & Social Sciences* 2(1): 23-25.

Imran, M., Bano, S., Nazir, S., Javid, A., Asad, M.J. and Yaseen, A. (2019). Cellulases Production and Application of Cellulases and Accessory Enzymes in Pulp and Paper Industry: A Review. *PSM Biological Research* 4(1): 29-39.

Irfan, M., Nadeem, M. and Syed, Q. (2012). Media optimization for amylase production in solid state fermentation of wheat bran by fungal strains. *Journal of Cell and Molecular. Biology* 10(1):55-64.

Irshad, M., Asgher, M., Bhatti, K.H., Zafar, M. and Anwar, Z. (2017). Anticancer and Nutraceutical Potentialities of Phytase/Phytate. *International Journal of Pharmacology* 13(7): 808-817.

Ja'afaru, M.I. (2013). Screening of fungi isolated from environmental samples for xylanase and cellulase production. *International Scholarly Research Notices: Microbiology* 2013: 7.

Jahangeer, S., Khan, N., Jahangeer, S., Sohail, M., Shahzad, S., Ahmad, A. and Khan, S.A. (2005). Screening and characterization of fungal cellulases isolated from the native environmental source. *Pakistan Journal of Botany* 37(3): 739.

Jamir, B. and Deb, C.R. (2014). Studies on some fermented foods and beverages of Nagaland, India. *International Journal of Fermented Foods* 3(2): 127.10.5958/2321-7111.2014.00001.8.

Jamir, N.S. and Rao, R.R. (1990). Fifty new or interesting medicinal plants used by the Zeliang of Nagaland (India). *Ethnobotany* 2(1): 11-18.

Jatuwong, K., Suwannarach, N., Kumla, J., Penkhrue, W., Kakumyan, P. and Lumyong, S. (2020) Bioprocess for production, characteristics, and biotechnological applications of fungal phytases. *Frontiers in Microbiology* 11:188. doi: 10.3389/fmicb.2020.00188.

Jayasiri, S.C., Hyde, K.D., Ariyawansa, H.A., Bhat, J., Buyck, B., Cai, L., Dai, Y.C., Abd-Elsalam, K.A., Ertz, D., Hidayat, I., Jeewon, R. and Jones, E.B.G. (2015). The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity* 74(1): 3-18.

Jeya, M., Zhang, Y.W., Kim, I.W. and Lee, J.K. (2009). Enhanced saccharification of alkali-treated rice straw by cellulase from *Trametes hirsuta* and statistical optimization of hydrolysis conditions by RSM. *Bioresource Technology* 100(21): 5155-5161.

Jeyaram, K., Singh, W., Capece, A. and Romano, P. (2008). Molecular identification of yeast species associated with "Hamei" — A traditional starter used for rice wine production in Manipur, India. *International Journal of Food Microbiology* 124(2): 115–125.

Jeyaram, J., Anand Singh, Th., Romi, W., Ranjita Devi, A., Mohendro Singh, W., Dayanidhi, H., Rajmuhon Singh, N. and Tamang, J.P. (2009). Traditional fermented foods of Manipur. *Indian Journal of Traditional Knowledge* 8 (1): 115-121.

Jeyaram, K., Tamang, J.P., Capece, A. and Romano, P. (2011). Geographical markers for Saccharomyces cerevisiae strains with similar technological origins

domesticated for rice-based ethnic fermented beverages production in North East India. *Antonie Van Leeuwenhoek* 100: 569–578.

Jian-rong, L. and Dang, L. (2006). The Application of Laccase (EC 1.10. 3.2) in Food Industry. *Modern Food Science and Technology* 4: 086.

Ji, Z., Jin, J., Yu, G., Mou, R., Mao, J., Liu, S., Zhou, Z. and Peng, L. (2018). Characteristic of filamentous fungal diversity and dynamics associated with wheat Qu and the traditional fermentation of Chinese rice wine. *International Journal of Food Science and Technology* 53(7): 1611-1621.

Joshi, V.K. (2016). *Indigenous fermented foods of South Asia*. Boca Raton, F.L: CRC press.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and Donoghue, M.J. (2002). Plant Systematics: A Phylogenetic Approach. 2nd ed. Sinauer Associates, Inc., Sunderland: 1-39.

Jurjevic, Z., Peterson, S.W. and Horn, B.W. (2012). *Aspergillus* section Versicolores: nine new species and multilocus DNA sequence based phylogeny. *IMA Fungus* 3(1): 59-79.

Jun, H., Kieselbach, T. and Jönsson, L.J. (2011). Enzyme production by filamentous fungi: analysis of the secretome of *Trichoderma reesei* grown on unconventional carbon source. *Microbial Cell Factories* 10(1): 68. doi.org/10.1186/1475-2859-10-68.

Jung, M.J., Nam, Y.D., Roh, S.W. and Bae, J.W. (2012). Unexpected convergence of fungal and bacterial communities during fermentation of traditional Korean alcoholic beverages inoculated with various natural starters. *Food Microbiology* 30 (1): 112-123.

Kameshwar, A.K.S. and Qin, W. (2019). Systematic review of publicly available non-Dikarya fungal proteomes for understanding their plant biomass-degrading and bioremediation potentials. *Bioresources and Bioprocessing* 6(1): 30. doi.org/10.1186/s40643-019-0264-6.

Kamil, O.H., Lupuliasa, D., Draganescu, D. and Vlaia, L. (2011). Interrelations of drying heat and survival of different fungal spores within the tablets formulation. *Studia Universitatis" Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series)* 21(2): 339.

Kanwar, S.S. and Bhushan, K. (2020). Ethnic fermented foods and beverages of Himachal Pradesh. In: *Ethnic Fermented Foods and Alcoholic Beverages of India:*

Science History and Culture (Ed: Tamang, J.P.), pp. 189-208. Springer Nature, Singapore.

Karimi, K. and Zamani, A. (2013). *Mucor indicus*: biology and industrial application perspectives: a review. *Biotechnology Advances* 31(4): 466-481.

Karlovsky, P., Suman, M., Berthiller, F., Meester, J.D., Eisenbrand, G., Perrin, I., Oswald, I.P., Speijers, G., Chiodini, A., Recker, T. and Dussort, P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*. 32(4): 179-205.

Khapudang, R., Sharma, S. and Joshi, V.K. (2018). Indigenous Fermented Food Products of Eastern and Western Himalayan Region: A review. *International Journal of Food and Fermentation Technology* 8(2): 119-139.

Khokhar, I., Mukhtar, I. and Mushtaq, S. (2011). Comparative Studies on the Amylase and Cellulase Production of *Aspergillus* and *Penicillium*. *Journal of Applied Sciences and Environmental Management* 15(4):657-661.

Kiefer, A.M., Seney, C.S., Lambright, A.L., Cottrill, K.A. and Young, V.A. (2018). Makgeolli: rapid production of an alcoholic beverage from the fermentation of rice. *Journal of Microbiology & Biology Education* 19(2): 19.2.77. doi.org/10.1128/jmbe.v19i2.1572.

Kim, H.R., Kim, J.H., Bai, D.H. and Ahn, B.H. (2011). Identification and characterization of useful fungi with α-amylase activity from the Korean traditional nuruk. *Mycobiology* 39(4): 278-282.

Kim, S.H., Lee, S.H., Yeo, S.H., Lee, S.H. and Cheong, C. (2017). Characterization of brewing microorganisms isolated from Korean traditional nuruk for Cheongju production. *Food Science and Biotechnology* 26(4): 959-967.

Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. (2013). *Ainsworth & Bisby's Dictionary of the Fungi,* 10th Ed. CABI Publishing, Wallingford.

Kolawole, O.M., Kayode, R.M.O. and Akinduyo, B. (2007). Proximate and microbial analyses of burukutu and pito produced in Ilorin, Nigeria. *African Journal of Biotechnology* 6(5): 587.

Kotaka, A., Bando, H., Kaya, M., Kato-Murai, M., Kuroda, K., Sahara, H. and Ueda, M. (2008). Direct ethanol production from barley β-glucan by sake yeast displaying *Aspergillus oryzae* β-glucosidase and endoglucanase. *Journal of bioscience and bioengineering*105(6): 622-627.

Krachunov, M., Nisheva, M. and Vassilev, D. (2017). Machine learning models in error and variant detection in high-variation high-throughput sequencing datasets. *Procedia Computer Science* 108: 1145-1154.

Kristbergsson, K. and Otles, S. (2016). Functional properties of traditional foods (Vol. 2). Springer.

Kuhad, R.C., Gupta, R. and Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme research* 2011: 10. doi.org/10.4061/2011/280696.

Kumar, D.S. and Ray, S. (2014). Fungal lipase production by solid state fermentation-an overview. *Journal of Analytical and Bioanalytical Techniques* 6(230): 1-10.

Kumar, S. and Chakravarty, S. (2018). Amylase. In *Enzymes in human and animal nutrition: principles and perspectives* (Eds: Nunes, C.S. and Kumar, V.), pp.163-182. Academic Press.

Kumar, V.A., Kurup, R.S.C., Snishamol, C. and Prabhu, G.N. (2019). Role of Cellulases in Food, Feed, and Beverage Industries. In *Green Bio-processes,* pp. 323-343. Springer, Singapore.

Kumar, S.S., Sreekumar, R. and Sabu, A. (2019a). Tannase and Its Applications in Food Processing. In *Green Bio-processes* pp. 357-381. Springer, Singapore.

Kumbhare, S.V., Dhotre, D.P., Dhar, S.K., Jani, K., Apte, D.A., Shouche, Y.S. and Sharma, A. (2015). Insights into diversity and imputed metabolic potential of bacterial communities in the continental shelf of Agatti Island. *Plos One* 10: 1-14.

Kurtzman, C.P. and Robnett, C.J. (2003). Phylogenetic relationships among yeasts of the 'Saccharomyces complex' determined from multigene sequence analyses. *FEMS Yeast Research* 3(4): 417-432.

Kutyauripo, J., Parawira, W., Tinofa, S., Kudita, I. and Ndengu, C. (2009). Investigation of shelf-life extension of sorghum beer (Chibuku) by removing the second conversion of malt. *International Journal of Food Microbiology* 129(3): 271-276.

Lahouar, A., Marin, S., Crespo-Sempere, A., Saïd, S. and Sanchis, V. (2016). Effects of temperature, water activity and incubation time on fungal growth and aflatoxin B1 production by toxinogenic *Aspergillus flavus* isolates on sorghum seeds. *Revista Argentina de Microbiologia* 48(1): 78-85.

Lai, X., Zhang, H., Liu, R. and Liu, C. (2015). Potential for aflatoxin B1 and B2 production by *Aspergillus flavus* strains isolated from rice samples. *Saudi Journal of Biological Science* 22(2): 176-180.

Laich, F., Fierro, F. and Martín, J.F. (2002). Production of penicillin by fungi growing on food products: identification of a complete penicillin gene cluster in *Penicillium griseofulvum* and a truncated cluster in *Penicillium verrucosum*. *Applied and Environmental Microbiology* 68(3): 1211-1219.

Lanka, S. and Latha, J.N.L. (2015). A short review on various screening methods to isolate potential lipase producers: lipases-the present and future enzymes of biotech industry. *International Journal of Biological Chemistry* 9(4): 207-219.

Lappe-Oliveras, P., Moreno-Terrazas, R., Arrizón-Gaviño, J., Herrera-Suárez, T., García-Mendoza, A., and Gschaedler-Mathis, A. (2008). Yeasts associated with the production of Mexican alcoholic non-distilled and distilled Agave beverages. *FEMS Yeast Research* 8(7): 1037-1052.

Laranjo, M., Potes, M.E. and Elias, M. (2019). Role of starter cultures on the safety of fermented meat products. *Frontiers in Microbiology* 10: 853. doi.org/10.3389/fmicb.2019.00853.

Leck, A. (1999). Preparation of lactophenol cotton blue slide mounts. *Community Eye Health* 12(30): 24.

Lee, S.W. (1984). Hankuk Sikpum Saltoesa (History of Korean Food and Society). *Kyomunsu. Seoul. Korea* 168.

Lee, A.C. and Fujio, Y. (1999). Microflora of banh men, a fermentation starter from Vietnam. *World Journal of Microbiology and Biotechnology* 15(1): 51-55.

Lee, C.H. and Kim, M.L. (2016). History of fermented foods in Northeast Asia. In: *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (Ed: Tamang, J.P.), pp. 1-16. Springer, New Delhi.

Lee, D.H., Choi, S.U. and Hwang, Y.I. (2005). Culture conditions and characterizations of a new phytase-producing fungal isolate, *Aspergillus sp.* L117. *Mycobiology* 33(4): 223-229.

Lee, C.H. and Lee, S.S. (2002). Cereal fermentation by fungi. In *Applied mycology and biotechnology* (Eds: Khachatourians, G.G. and Arora, D.K.) (Vol. 2), pp:151-170. Elsevier.

Lee, S.C., Billmyre, R.B., Li, A., Carson, S., Sykes, S.M., Huh, E.Y., Mieczkowski, P., Ko, D.C., Cuomo, C.A. and Heitman, J. (2014). Analysis of a food-borne fungal pathogen outbreak: virulence and genome of a *Mucor circinelloides* isolate from yogurt. *mBio* 5(4): e01390-14. doi.org/10.1128/mBio.01390-14.

Lei, X.G., Weaver, J.D., Mullaney, E., Ullah, A.H. and Azain, M.J. (2013). Phytase, a new life for an "old" enzyme. *Annual Review of Animal Bioscience* 1(1)*:* 283-309.

Leroy, F. and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology* 15(2): 67-78.

Leyva Salas, M., Mounier, J., Valence, F., Coton, M., Thierry, A. and Coton, E. (2017). Antifungal microbial agents for food biopreservation—a review. *Microorganisms* 5(3): 37. doi: 10.3390/microorganisms5030037.

Li, Y., Wadsö, L. and Larsson, L. (2009). Impact of temperature on growth and metabolic efficiency of *Penicillium roqueforti*–correlations between produced heat, ergosterol content and biomass. *Journal of Applied Microbiology* 106(5): 1494-1501.

Li, Y., Zheng, X., Zhang, X., Bao, L., Zhu, Y., Qu, Y., Zhao, J. and Qin, Y. (2016). The different roles of *Penicillium oxalicum* LaeA in the production of extracellular cellulase and β-xylosidase. *Frontiers in Microbiology* 7 2091. doi: 10.3389/fmicb.2016.02091.

Lima, J.S.D., Cruz, R., Fonseca, J.C., Medeiros, E.V.D., Maciel, M.D.H.C., Moreira, K.A. and Motta, C.M.D.S. (2014). Production, characterization of tannase from *Penicillium montanense* URM 6286 under SSF using agroindustrial wastes, and application in the clarification of grape juice (*Vitis vinifera* L.). *The Scientific World Journal* 2014: 9. doi.org/10.1155/2014/182025.

Limtong, S., Sintara, S., Suwanarit, P. and Lotong, N. (2002). Yeast diversity in Thai traditional fermentation starter (Loog-pang). *Kasetsart Journal: Natural Science* 36: 149-158.

Liu, T., Kang, J., Liu, L., Hu, X., Wang, X., Li, X., Ma, Z. and Ren, T. (2020). Microbial community diversity of traditional dough starter (*Jiaozi*) from two provinces in northwest China. *Annals of Microbiology* 70: 18 (2020). doi.org/10.1186/s13213-020-01544-1.

Londoño-Hernández, L., Ramírez-Toro, C., Ruiz, H.A., Ascacio-Valdés, J.A., Aguilar-Gonzalez, M.A., Rodríguez-Herrera, R. and Aguilar, C.N. (2017). *Rhizopus oryzae* – ancient microbial resource with importance in modern food industry. *International Journal of Food Microbiology* 257: 110-127.

Lu, R. and Miyakoshi, T. (2015). Laccase. *Lacquer Chemistry and Applications* 95–104.

Lucas. R., Groeneveld, J., Harms, H., Johst, K., Frank, K. and Kleinsteuber, S. (2017). A critical evaluation of ecological indices for the comparative analysis of microbial communities based on molecular datasets. *FEMS Microbiology Ecology* 93(1). doi.org/10.1093/femsec/fiw209.

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B. and Grube, M. (2004). Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91: 1446-1480.

Lv, X.C., Huang, Z.Q., Zhang, W., Rao, P.F. and Ni, L. (2012a). Identification and characterization of filamentous fungi isolated from fermentation starters for Hong Qu glutinous rice wine brewing. *The Journal General and Applied Microbiol*ogy 58: 33-42.

Lv, X.C., Weng, X., Zhang, W., Rao, P.F. and Ni, L. (2012b). Microbial diversity of traditional fermentation starters for Hong Qu glutinous rice wine as determined by PCR-mediated DGGE. *Food Control* 28(2), 426-434.

Lv, X.C., Huang, X.L., Zhang, W., Rao, P.F. and Ni, L. (2013). Yeast diversity of traditional alcohol fermentation starters for Hong Qu glutinous rice wine brewing, revealed by culture-dependent and culture-independent methods. *Food Control* 34(1): 183-190.

Lv, X.C., Cai, Q.Q., Ke, X.X., Chen, F., Rao, P.F. and Ni, L. (2015). Characterization of fungal community and dynamics during the traditional brewing of Wuyi Hong Qu glutinous rice wine by means of multiple culture-independent methods. *Food Control* 54: 231-239.

Ly, S., Mith, H., Tarayre, C., Taminiau, B., Daube, G., Fauconnier, M.L. and Delvigne, F. (2018). Impact of microbial composition of Cambodian traditional dried starters (Dombea) on flavor compounds of rice wine: combining amplicon sequencing with HP-SPME-GCMS. *Frontiers in Microbiol*ogy 9: 894. doi: 10.3389/fmicb.2018.00894.

Ma, R., Sui, L., Zhang, J., Hu, J. and Liu, P. (2019). Polyphasic characterization of yeasts and lactic acid bacteria metabolic contribution in semi-solid fermentation of Chinese baijiu (traditional fermented alcoholic drink): towards the design of a tailored starter culture. *Microorganisms* 7: 147. doi.org/10.3390/microorganisms705014.

Mahdihassan, S. (1979). Distillation assembly of pottery in ancient India with a single item of special construction. *Visvesvaran and Indological Journal* 17: 264.

Mahdihassan, S. (1981). Parisrut the earliest distilled liquor of Vedic times or of about 1500 BC. *Indian Journal of History of Science* 16(2): 223-229.

Mamo, J. and Assefa, F. (2018). The role of microbial aspartic protease enzyme in food and beverage industries. *Journal of Food Quality* 2018. doi.org/10.1155/2018/7957269.

Mani, A. (2018). Food Preservation by Fermentation and Fermented food products. *International Journal of Academic Research & Development*. 51-57.

Marsh, A., Hill, C., Ross, R. and Cotter, P. (2014). Fermented beverages with health-promoting potential: Past and future perspectives. *Trends in Food Science and Technology* 38: 10. doi.1016/j.tifs.2014.05.002.

Martin, K.J. and Rygiewicz, P.T. (2005). Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC Microbiology* 5: 28.doi.org/10.1186/1471-2180-5-28.

Martin, M.F., Okpo, E.A. and Andy, I.E. (2019). Microbial amylases: A review. World *News of Natural Sciences* 22: 174-179.

Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G. and Neufeld, J.D. (2012). PANDAseq: paired-end assembler for illumina sequences. *BMC bioinformatics* 13(1): 31.

McGinnis, M.R. and Tyring, S.K. (1996). Introduction to mycology. *Medical Microbiology*. University of Texas Medical Branch at Galveston, Galveston, TX, https://www. ncbi. nlm. nih. gov/books/NBK8471/McKone, TE, 165-181.

McKelvey, S.M. and Murphy, R.A. (2011). Biotechnological use of fungal enzymes. *Biology and Applications* 179. doi.org/10.1002/9781119374312.ch8.

Mehta, A., Bodh, U. and Gupta, R. (2017). Fungal lipases: a review. *Journal of Biotech Research* 8.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry* 31(3): 426-428.

Mishra, B.K., Hati, S., Brahma, J., Patel, M. and Das, S. (2018). Identification and Characterization of Yeast Strains Associated with the Fermented Rice Beverages of Garo Hills, Meghalaya, India. *International Journal of Current Microbiology and Applied Sciences*7(2): 3079-3090.

Mishra, B.K., Hati, S., Das, S. and Brahma, J. (2019). Fermented rice beverage of Northeast India: A systematic review. *International Journal of Fermented Foods* 8(1): 41-56.

Molino, S., Casanova, N.A., Rufián, J.H. and Fernandez, M.M. (2019). Natural Tannin Wood Extracts as a Potential Food Ingredient in the Food Industry. *Journal of Agricultural and Food Chemistry* doi: 10.1021/acs.jafc.9b00590.

Moore, D. and Chiu, S.W. (2001). Fungal products as food. In *Bio-Exploitation of Filamentous Fungi* (Ed: Pointing, S.B. and Hyde, K.D.), pp. 223-251. *Fungal Diversity* Press: Hong Kong.

Moreira, K.A., Herculano, P.N., Maciel, M.H.C., Porto, T.S., Spier, M.R., Souza-Motta, C.M., Porto, A.L.F. and Soccoi, C.R. (2014). Optimization of phytase production by Aspergillus japonicus Saito URM 5633 using cassava bast as substrate in solid state fermentation. *African Journal of Microbiology Research* 8(9): 929-938.

Mota de Carvalho, N., Costa, E.M., Silva, S., Pimentel, L., Fernandes, T.H. and Pintado, M.E. (2018). Fermented foods and beverages in human diet and their influence on gut microbiota and health. *Fermentation* 4(4): 90. doi.org/10.3390/fermentation4040090.

Mudili, V., Siddaih, C.N., Nagesh, M., Garapati, P., Naveen Kumar, K., Murali, H.S., Mattila, T.Y. and Batra, H.V. (2014). Mould incidence and mycotoxin contamination in freshly harvested maize kernels originated from India. *Journal of the Science Food and Agriculture* 94(13): 2674-2683.

Nakazato, M., Morozumi, S., Saito, K., Fujinuma, K., Nishima, T. and Kasai, N. (1990). Interconversion of aflatoxin B1 and aflatoxicol by several fungi. *Applied and Environmental Microbiology* 56(5): 1465-1470.

Nkhata, S.G., Ayua, E., Kamau, E.H. and Shingiro, J.B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science and Nutrition* 6(8): 2446-2458.

Nandy, S.N., Dhyani, P.P. and Sanal, P.K. (2006). Resources information database of the Indian Himalaya. *ENVIS Monograph* 3: 1-95.

Naranjo-Ortiz, M.A. and Gabaldón, T. (2019). Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. *Biological Reviews* 94(6): 2101-2137.

Narzary, Y., Brahma, J., Brahma, C. and Das, S. (2016). A study on indigenous fermented foods and beverages of Kokrajhar, Assam, India. *Journal of Ethnic Foods* 3(4): 284-291.

Nath, N., Ghosh, S., Rahaman, L., Kaipeng, D.L. and Sharma, B.K. (2019). An overview of traditional rice beer of North-east India: ethnic preparation, challenges and prospects. *Indian Journal of Traditional Knowledge* 18(4): 744-757.

Naumann, H.D., Tedeschi, L.O., Zeller, W.E. and Huntley, N.F. (2017). The role of condensed tannins in ruminant animal production: advances, limitations and future directions. *Revista Brasileira de Zootecnia* 46(12): 929-949.

Nehal, N. (2013). Knowledge of traditional fermented food products harbored by the tribal folks of the Indian Himalayan belt. *International Journal of Agriculture and Food Science Technology* 4(5): 401-414.

Nikkuni, S., Karki, T.B., Terao, T. and Suzuki, C. (1996). Microflora of mana, a Nepalese rice koji. *Journal of Fermentation and Bioengineering* 81(2): 168-170.

Nile, S.H. (2015). The nutritional, biochemical and health effects of makgeolli – a traditional Korean fermented cereal beverage. *Journal of Institute of Brewing* 121: 457–463.

Nout, M.J.R. (1995). Fungal interactions in food fermentations. *Canadian Journal of Botany* 73(S1): 1291-1300.

Nout, M.J.R. and Aidoo, K.E. (2011). Asian fungal fermented food. In Industrial applications (pp. 29-58). Springer, Berlin, Heidelberg.

Nout, M.J.R. and Motarjemi, Y. (1997). Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. *Food Control* 8(5-6): 221-226.

Nout, M.J.R. and Sarkar, P.K. (1999). Lactic acid food fermentation in tropical climates. In *Lactic Acid Bacteria: Genetics, Metabolism and Applications* (Eds: Konings, Wil., Kuipers, O.P., Huis in 't Veld, J.H.J). (pp. 395-401). Springer, Dordrecht.

Nout, M.J.R. and Aidoo, K.E. (2002). Asian fungal fermented food. In: *The Mycota* (Ed: Osiewacz, H.D.), pp. 23-47. Springer-Verlag, New York.

Nuraida, L. 2015. A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. *Food Science and Human Wellness* 4(2):47–55.

Nuraida, L. and Krusong, W. (2015). Starter cultures. In: "Indigenous Fermented Foods of Southeast Asia". (Ed. Owens, J.D.). New York. CRC Press.

Nwuche, C.O. and Ogbonna, J.C. (2011). Isolation of lipase producing fungi from palm oil mill effluent (POME) dump sites at Nsukka. *Brazilian Archives of Biology and Technology* 54(1): 113-116.

Odoni, D.I., Tamayo-Ramos, J.A., Sloothaak, J., van Heck, R.G.A., Martins Dos Santos, V.A.P., de Graaff, L.H., Suarez-Diez, M. and Schaap, P.J. (2017). Comparative proteomics of *Rhizopus delemar* ATCC 20344 unravels the role of amino acid catabolism in fumarate accumulation. *Peer J the Journal of Life and Environmental Sciences* 5: e3133. doi: 10.7717/peerj.3133.

Ohba, R., Koga, T. and Ueda, S. (1989). Liquefaction of glutinous rice and aroma formation in tapé preparation by ragi. *Journal of Fermentation and Bioengineering* 67(4): 249-252.

Okafor, U.A., Emezue, N.T., Okochi, V.I., Onyegeme-Okerenta, B.M. and Chinedu, S.N. (2007). Xylanase production by *Penicillium chrysogenum* (PCL501) fermented on cellulosic wastes. *African Journal of Biochemistry Research* 1(4): 048-053.

Okpara, M.O., Ba-midele, O.S. and Ajele, J.O. (2019) Enhanced Production of Salinity-Induced Proteases from *Aspergillus flavus* and *Aspergillus niger*. *Advances in Enzyme Research* 7: 45-56.

Olempska-Beer, Z.S., Merker, R.I., Ditto, M.D. and DiNovi, M.J. (2006). Food-processing enzymes from recombinant microorganisms—a review. *Regulatory Toxicology and Pharmacology* 45(2): 144-158.

Ogunremi, O.R., Banwo, K. and Sanni, A.I. (2017). Starter-culture to improve the quality of cereal-based fermented foods: trends in selection and application. *Current Opinion in Food Science* 13: 38-43.

Omemu, A.M., Bamigbade, G., Obadina, A.O. and Obuotor, T.M. (2015). Isolation and Screening of Amylase from Moulds Associated with the Spoilage of Some Fermented Cereal Foods. *Microbiology Research Journal International* 359-367.

Østergaard, L.H. and Olsen, H.S. (2011). Industrial applications of fungal enzymes. In *Industrial applications* (Ed: Martin, H.), pp. 269-290. Springer, Berlin, Heidelberg.

Owens, J.D. (2014). Indigenous fermented foods of Southeast Asia. CRC Press.

Oyeleke, S.B., Egwim, E.C. and Auta, S.H. (2010). Screening of *Aspergillus flavus* and *Aspergillus fumigatus* strains for extracellular protease enzyme production. *Journal of Microbiology and Antimicrobials* 2(7): 83-87.

Padhiar, A.R. and Kommu, S. (2016). Isolation, Characterization and Optimization of Bacteria producing Amylase. *International Journal of Advanced Research in Biological Sciences*3(7):1-7.

Palacios-Cabrera, H., Taniwaki, M.H., Hashimoto, J.M. and Menezes, H.C.D. (2005). Growth of *Aspergillus ochraceus, A. carbonarius* and *A. niger* on culture media at different water activities and temperatures. *Brazilian Journal of Microbiology* 36(1): 24-28.

Palaniswamy, M., Pradeep, B.V., Sathya, R. and Angayarkanni, J. (2008). Isolation, identification and screening of potential xylanolytic enzyme from litter degrading fungi. *African Journal of Biotechnology* 7:(12). doi.org/10.5897/AJB2008.000-5045.

Panda, T., Pani, P.K., Mishra, N. and Mohanty, R.B. (2010). A comparative account of the diversity and distribution of fungi in tropical forest soils and sand dunes of Orissa, India. *Journal of. Biodiversity*. 1(1): 27-41.

Pandey, N.K., Chhonkar, D.S., Singh, D.K. and Khumu, S.T. (2017). Analysis of Indigenous Food Items of Monpa Tribal Community in Tawang District of Arunachal Pradesh, India. *International Journal of Current Microbiology and Applied Sciences* 6(9): 633-640.

Parija, S.C. and Prabhakar, P.K. (1995). Evaluation of lacto-phenol cotton blue for wet mount preparation of feces. *Journal of Clinical Microbiology* 33(4): 1019-1021.

Park, K.H., Liu, Z., Park, C.S. and Ni, L. (2016). Microbiota associated with the starter cultures and brewing process of traditional Hong Qu glutinous rice wine. *Food Science and Biotechnology* 25(3): 649-658.

Park, H.S., Jun, S.C., Han, K.H., Hong, S.B. and Yu, J.H. (2017). Diversity, application, and synthetic biology of industrially important *Aspergillus* fungi. *In Advances in Applied Microbiology* (Vol. 100) (Eds: Gadd, G.M. and Sariaslani, S.), pp. 161-202. Academic Press.

Parveen, B.T.N. and Manikandaselvi, S. (2011). Production of Lipase enzyme by *Aspergillus flavus* using Groundnut waste. *International Journal of PharmTech Research* 3(3) 1299-1302.

Patel, S.J. and Savanth, V.D. (2015). Review on fungal xylanases and their applications. *International Journal of Advanced Research* 3(3): 311-315.

Patro, K.R., Basak, U.C., Mohapatra, A.K. and Gupta, N. (2014). Development of new medium composition for enhanced production of L-asparaginase by *Aspergillus flavus*. *Journal of Environmental Biology* 35: 295-300.

Payne, C.M., Knott, B.C., Mayes, H.B., Hansson, H., Himmel, M.E., Sandgren, M., Stahlberg, J. and Beckham, G.T. (2015). Fungal cellulases. *Chemical Reviews* 115(3): 1308-1448.

Peele, S. (1997). Utilizing culture and behavior in epidemiological models of alcohol consumption and consequences for Western nations. *Alcohol & Alcoholism* 32: 51-64.

Pierce, G. and Toxqui, Á. (2014). *Alcohol in Latin America: A Social and Cultural History.* University of Arizona Press, Tucson, Arinona, USA.

Pietikäinen, J., Pettersson, M. and Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology* 52(1): 49-58.

Pinto, F.C.J., Lima, D.B.D., Agustini, B.C., Dallagassa, C.B., Shimabukuro, M.F., Chimelli M. and Bonfim, T.M.B. (2012). Morphological and molecular identification of filamentous fungi isolated from cosmetic powders. *Brazilian Archives of Biology and Technology* 55: 897-901.

Piontek, M., Łuszczyńska, K. and Lechów, H. (2016). Occurrence of the toxin-producing *Aspergillus versicolor* Tiraboschi in residential buildings. *International Journal of Environmental Research and Public Health* 13(9): e862. doi: 10.3390/ijerph13090862.

Pires, E.B.E., de Freitas, A.J., e Souza, F.F., Salgado, R.L., Guimarães, V.M., Pereira, F.A. and Eller, M.R. (2019). production of Fungal phytases from Agroindustrial Byproducts for pig Diets. *Scientific Reports* 9(1): 1-9.

Pitt. J.I. and Hocking, A.D. (2009). Fungi and food spoilage (3rd ed.). New York: Springer Dordrecht.

Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A. and Amorim, D.S. (2005). Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology* 67(5): 577-591.

Pradhan, P. and Tamang, J.P. (2019). Phenotypic and genotypic identification of bacteria isolated from traditionally prepared dry starters of the Eastern Himalayas. *Frontiers in Microbiology* 10:2526. doi: 10.3389/fmicb.2019.02526.

Prakash, O. (1961). Food and drinks in ancient India. (Delhi): Munshi Ram Monoharial Publication.

Prigione, V., Spina, F., Tigini, V., Giovando, S. and Varese, G.C. (2018). Biotransformation of industrial tannins by filamentous fungi. *Applied Microbiology and Biotechnology* 102(24): 10361-10375.

Priyanka, S.R., Ramana, M.V., Balakrishna, K., Murali, H.S. and Batra, H.V. (2012). A novel non radioactive PCR-DNA probe for the detection of aflatoxin producing *Aspergillus* species from major food crops grown in India. *Advances in Microbiology* 577-586.

Qiao, W., Tao, J., Luo, Y., Tang, T., Miao, J. and Yang, Q. (2018). Microbial oil production from solid-state fermentation by a newly isolated oleaginous fungus, *Mucor circinelloides* Q531 from mulberry branches. *Royal Society Open Science* 5(11): 180551. doi: 10.1098/rsos.180551.

Quan, C., Zhang, L., Wang., Y. and Ohta, Y. (2001). Production of phytase in a low phosphate medium by a novel yeast *Candida krusei*. *Journal of Bioscience and Bioengineering* 92(2):154-60.

Quiroz-Castañeda, R.E., Balcázar-López, E., Dantán-González, E., Martinez, A., Folch-Mallol, J. and Martínez Anaya, C. (2009). Characterization of cellulolytic activities of *Bjerkandera adusta* and *Pycnoporus sanguineus* on solid wheat straw medium. *Electronic Journal of Biotechnology* 12(4). DOI: 10.2225/vol12-issue4-fulltext-3.

Rai, B.K. and Subba, D. K. (2016). "Basic Practical Manual on Industrial Microbiology" (1st ed.). Science and Technology Education Service (NAAST), Nepal.

Raja, H.A., Miller, A.N., Pearce, C.J. and Oberlies, N.H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. *Journal of Natural Products* 80: 756-770.

Rajan, A., Kumar, D.R. and Nair, A. (2011), “Isolation of a novel alkaline lipase producing fungus *Aspergillus fumigates* MTCC 9657 from aged and crude and rice bran oil and quantification by HPTLC”. *International Journal of Biological Chemistry* 5(2): 116-126.

Ram, L., Kaur, K. and Sharma, S. (2014). Screening isolation and characterization of cellulase producing microorganisms from soil. *International Journal of Pharmaceutical Science Invention* 3(3): 12-18.

Ramachandran, S., Roopesh, K., Nampoothiri, K.M., Szakacs, G. and Pandey, A. (2005). Mixed substrate fermentation for the production of phytase by *Rhizopus* spp. using oilcakes as substrates. *Process Biochemistry* 40(5): 1749-1754.

Ramanjaneyulu, G., Reddy, G.P.K., Kumar, K.D. and Reddy, B.R. (2015). Isolation and screening of xylanase producing fungi from forest soils. *International Journal of Current Microbiology Applied Sciences* 4: 586-591.

Ramírez-Camejo, L.A., Zuluaga-Montero, A., Lázaro-Escudero, M.A., Hernández-Kendall, V. N. and Bayman, P. (2012) Phylogeography of the cosmopolitan fungus *Aspergillus flavus*: is everything everywhere?". *Fungal Biology* 116 (3): 452–463.

Ramnath, L., Sithole, B. and Govinden, R. (2017). Identification of lipolytic enzymes isolated from bacteria indigenous to Eucalyptus wood species for application in the pulping industry. *Biotechnology Reports* 15: 114-124.

Rana, T.S., Datt, B. and Rao, R.R. (2004). Soor: a traditional alcoholic beverage in Tons valley, Garhwal Himalaya. *Indian Journal of Traditional Knowledge* 3(1): 59-65.

Rathi, V. (2018). Herbal wine: a review. *Journal of Nutrition and Weight Loss* 3: 113. doi: 10.4172/2475-3181.1000113.

Raveendran, S., Parameswaran, B., Ummalyma, S.B., Abraham, A., Kuruvilla Mathew, A., Madhavan, A., Rebello S. and Pandey, A. (2018). Applications of microbial enzymes in food industry. *Food Technology and Biotechnology* 56(1): 16-30.

Ray, M., Ghosh, K., Singh, S. and Mondal, K.C. (2016). Folk to functional: an explorative overview of rice-based fermented foods and beverages in India. *Journal of Ethnic Foods* 3(1): 5-18.

Razzaq, A., Shamsi, S., Ali, A., Ali, Q., Sajjad, M., Malik, A. and Ashraf, M. (2019). Microbial proteases applications. *Frontiers in Bioengineering and Biotechnology* 7. doi: 10.3389/fbioe.2019.00110.

Ribeiro, B.D., Castro, A.M.D., Coelho, M.A.Z. and Freire, D.M.G. (2011). Production and use of lipases in bioenergy: a review from the feedstocks to biodiesel production. *Enzyme Research* 2011:615803. doi: 10.4061/2011/615803.

Rodríguez Couto, S., Rodríguez, A., Paterson, R.R.M., Lima, N. and Teixeira, J.A. (2006). Laccase activity from the fungus *Trametes hirsuta* using an air-lift bioreactor. *Letters in Applied Microbiology* 42(6): 612-616.

Rolle, R. and Satin, M. (2002). Basic requirements for the transfer of fermentation technologies to developing countries. *International Journal of Food Microbiology* 75(3): 181-187.

Romanelli, A.M., Sutton, D.A., Thompson, E.H., Rinaldi, M.G. and Wickes, B.L. (2010). Sequence-based identification of filamentous basidiomycetous fungi from clinical specimens: a cautionary note. *Journal of Clinical Microbiology* 48(3):741-752.

Rosales, E., Couto, S.R. and Sanromán, M.A. (2005). Reutilisation of food processing wastes for production of relevant metabolites: application to laccase production by *Trametes hirsute. Journal of Food Engineering* 66(4): 419-423.

Roslan, R., Rehan, M.M., Kamarudin, K.R., Noor, H.M., Huda-Faujan, N. and Radzi, S.M. (2018). Isolation and identification of amylolytic bacteria from Ragi. *Malaysian Applied Biology Journal* 47(2): 83–88.

Roy, B., Kala, C.P., Farooquee, N.A. and Majila, B.S. (2004). Indigenous fermented food and beverages: a potential for economic development of the high altitude societies in Uttaranchal. *Journal of Human Ecology* 15(1): 45-49.

Saikia, B., Tag, H. and Das, A.K. (2007). Ethnobotany of foods and beverages among the rural farmers of Tai Ahom of North Lakhimpur district, Asom. *India. Journal of Traditional Knowledge* 6(1): 126-132.

Sakthiselvan, P., Naveena, B. and Partha, N. (2014). Molecular characterization of a Xylanase-producing fungus isolated from fouled soil. *Brazilian Journal of Microbiology* 45(4): 1293-1302.

Saleem, A. and Ebrahim, M.K. (2014). Production of amylase by fungi isolated from legume seeds collected in Almadinah Almunawwarah, Saudi Arabia. *Journal of Taibah University for Science* 8(2): 90-97.

Salihu, Y., Saidu, A.Y., Rabiu, G.A., Umar, A.A., Zeynep, A. and Unzile, G. (2015). Detection of Alpha-Amylase Activity from Soil Bacteria. *IOSR Journal of Biotechnology and Biochemistry* 1(6): 01-09.

Sajith, S., Priji, P., Sreedevi, S. and Benjamin, S. (2016). An overview on fungal cellulases with an industrial perspective. *Journal of Nutrition and Food Science* 6(1): 461. doi: 10.4172/2155-9600.1000461.

Samati, H. and Begum, S.S. (2007). *Kiad*-a popular local liquor of *Pnar* tribe of Jaintia hills district, Meghalaya. *Indian Journal of Traditional Knowledge* 6(1): 133-135.

Sambrook, J. and Russell, D.W. (2001). Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor Laboratory Press: New York.

Samson, R.A., Hoekstra, E.S. and Frisvad, J.C. (2004). Introduction to Food and Airborne Fungi. 7th ed. Central bureasuvoor Schimmer cultures (CBS); Utrecht, The Netherlands.

Samson, R.A., Visagie, C.M., Houbraken, J., Hong, S.B., Hubka, V., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Susca, A., Tanney, J.B., Varga, J., Kocsube, S., Szigeti, G., Yaguchi, T. and Frisvad, J.C. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in Mycology* 78: 141-173.

Samtiya, M., Aluko, R.E. and Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition* 2(1): 1-14.

Sanjotha, G. (2017). Isolation, screening, optimization Asparaginase by fungi from karwar coastal region. *Research Journal of Recent Sciences* 6(3): 1-7.

Şanlier, N., Gökcen, B.B. and Sezgin, A.C. (2019). Health benefits of fermented foods. *Critical Reviews in Food Science and Nutrition* 59(3): 506-527.

Saori, A. and Keller, N.P. (2011). *Aspergillus flavus*. *Annual Review Phytopathology* 49: 107–133.

Saranraj, P. and Stella, D. (2013). Fungal amylase—a review. *International Journal of Microbiological Research* 4(2): 203-211.

Sarkar, A., Mukherjee, A., Bera, M.K., Das, A., Juyal, N., Morthekai, R., Deshpande, R.D., Shinde, V.S. and Rao, K.S. (2016). Oxygen isotope in archaeological bioapatite from India: Implications to climate change and decline of Bronze Age Harappan civilization. *Scientific Reports* 6: 26555. doi: 10.1038/srep26555.

Sarquis, M.I.D.M., Oliveira, E.M.M., Santos, A.S. and Costa, G.L.D. (2004). Production of L-asparaginase by filamentous fungi. *Memorias do Instituto Oswaldo Cruz* 99(5): 489-492.

Savary, R., Villard, L. and Sanders, I.R. (2018). Within-species phylogenetic relatedness of a common mycorrhizal fungus affects evenness in plant communities through effects on dominant species. *Plos One* 13(11): e0198537. doi.org/10.1371/journal.pone.0198537.

Savic, M., Room, R., Mugavin, J., Pennay, A. and Livingston, M. (2016). Defining “drinking culture”: A critical review of its meaning and connotation in social

research on alcohol problems. *Drugs: Education, Prevention and Policy* 23(4): 270-282.

Saxena, J., Pant, V., Sharma, M.M., Gupta, S. and Singh, A. (2015). Hunt for Cellulase Producing Fungi from Soil Samples. *Journal of Pure and Applied Microbiology* 9(4): 2895-2902.

Schmidt-Dannert, C. (2016). Biocatalytic portfolio of Basidiomycota. *Current Opinion in Chemical Biology* 31: 40–49.

Schmitz, A. and Riesner, D. (2006). Purification of nucleic acids by selective precipitation with polyethylene glycol 6000. *Analytical Biochemistry* 354(2): 311-313.

Scott, R. and Sullivan, W.C. (2008). Ecology of fermented foods. Research in Human Ecology Review15 (1): 25-31.

Sharma, D., Sharma, B. and Shukla, A.K. (2011). Biotechnological approach of microbial lipase: a review. *Biotechnology* 10(1): 23-40.

Sharma, A.K., Sharma, V., Saxena, J., Yadav, B., Alam, A. and Prakash, A. (2015). Isolation and screening of extracellular protease enzyme from bacterial and fungal isolates of soil. *International Journal of Scientific Research in Environmental* 3(9): 0334-0340.

Sharma, M., Gat, Y., Arya, S., Kumar, V., Panghal, A. and Kumar, A. (2019). A Review on Microbial Alkaline Protease: An Essential Tool for Various Industrial Approaches. *Industrial Biotechnology* 15(2): 69-78.

Shenoy, B.D., Jeewon, R. and Hyde, K.D. (2007). Impact of DNA sequence-data on the taxonomy of anamorphic fungi. *Fungal Diversity* 26(2007): 1-54.

Senthivelan, T., Kanagaraj, J., Panda, R.C. and Narayani, T. (2019). Screening and production of a potential extracellular fungal laccase from *Penicillium chrysogenum*: media optimization by response surface methodology (RSM) and central composite rotatable design (CCRD). *Biotechnology Reports* 23 e00344. doi.org/10.1016/j.btre.2019.e00344.

Serna-Jimenez, J.A., Quintanilla-Carvajal, M.X., Rodriguez, J.M., Uribe, M.A. and Klotz, B. (2016). Development of a Combined Temperature and pH Model and the Use of Bioprotectants to Control of *Mucor circinelloides*. *American Journal of Food Technology* 11(1-2): 21-28.

Sha, S.P., Anupma, A., Pradhan, P., Prasad, G.S. and Tamang, J.P. (2016). Identification of yeasts by PCR-mediated DGGE in *marcha*, an ethnic amylolytic starter of India. *Journal of Ethnic Foods* 3: 292-296.

Sha, S.P., Jani, K., Sharma, A., Anupma, A., Pradhan, P., Shouche, Y. and Tamang, J.P. (2017). Analysis of bacterial and fungal communities in *Marcha* and *Thiat*, traditionally prepared amylolytic starters of India. *Scientific Reports* 7(1): 10967. doi:10.1038/s41598-017-11609-y.

Sha, S.P., Suryavanshi, M.S., Jani, K., Sharma, A., Shouche, Y.S. and Tamang, J.P. (2018). Diversity of yeasts and molds by culture-dependent and culture
independent methods for mycobiome surveillance of traditionally prepared dried starters for the production. *Frontier in Microbiology* 9: 2237. doi: 10.3389/fmicb. 2018.02237.

Sha, S.P., Suryavanshi, M.S. and Tamang, J.P. (2019). Mycobiome diversity in traditionally prepared starters for alcoholic beverages in India by high throughput sequencing method. *Frontier in Microbiology* 10: 348. doi: 10.3389/fmicb.2019.003482237.

Shabeena, A.K.S., Ravi, M. and Jayaraj, Y.M. (2017). Isolation and identification of xylanase producing fungal isolate. *International Journal of Advanced Research in Biological Sciences* 4(3): 102-106.

Shah, P.C., Kumar, V.R., Dastager, S.G. and Khire, J.M. (2017). Phytase production by *Aspergillus niger* NCIM 563 for a novel application to degrade organophosphorus pesticides. *AMB Express* 7(1): 66.doi: 10.1186/s13568-017-0370-9.

Shahriarinour, M., Wahab, M.N.A., Ariff, A. and Mohamad, R. (2011). Screening, isolation and selection of cellulolytic fungi from oil palm empty fruit bunch fibre. *Biotechnology* 10(1): 108-113.

Shakeel, Q., Lyu, A., Zhang, J., Wu, M., Li, G., Hsiang, T. and Yang, L. (2018). Biocontrol of *Aspergillus flavus* on peanut kernels using *Streptomyces yanglinensis* 3-10. *Frontier in Microbiology* 9: 1049.doi: 10.3389/fmicb.2018.01049

Sharifyazd, S. and Karimi, K. (2017). Effects of fermentation conditions on valuable products of ethanolic fungus *Mucor indicus*. *Electronic Journal of Biotechnology* 30: 77-82.

Shin, H.M., Lim, J.W., Shin, C.G. and Shin, C.S. (2017). Comparative characteristics of rice wine fermentations using *Monascus* koji and rice nuruk. *Food Science and Biotechnology* 26(5): 1349-1355.

Shrestha, H.N.K. and Rati E.R. (2002). Microbiological profile of *murcha* starters and physico-chemical characteristics of poko, a rice based traditional food products of Nepal. *Food Biotechnology* 16: 1–15.

Shrivastava, K., Greeshma, A.G. and Srivastava, B. (2012). Biotechnology in tradition – a process technology of alcoholic beverages practiced by different tribes of Arunachal Pradesh, North East India. *Indian Journal of Traditional Knowledge* 11(2): 81-89.

Shrivastava, K., Greeshma, A.G. and Srivastava, B. (2012a). Biotechnology in Tradition-Methods of starter preparation for alcoholic beverages employed by different tribal communities of Arunachal Pradesh. *Indian Journal of Traditional Knowledge* 11(1): 194-201.

Shoubao, Y., Xiangsong, C. and Jiaquan, G. (2019). Bacterial and fungal diversity in the traditional Chinese strong flavour liquor Daqu. *Journal of the Institute of Brewing* 125:443– 452.

Singaravadivel, K., Alagusundaram, K. and Hariharan, B. (2012). Physicochemical properties of fresh and stored coconut palm toddy. *Open Access Scientific Reports* 1(8). DOI: 10.4172/scientificreports.397.

Singh, P.K. and Singh, K.I. (2006). Traditional alcoholic beverage, Yu of Meitei communities of Manipur. *Indian Journal of Traditional Knowledge.* 5(2): 184-190.

Singh, R., Gupta, N., Goswami, V. and Gupta, R. (2006), “A simple activity staining protocol for lipases and esterases” *Applied Microbiology and Biotechnology* 70: 679–682.

Singh, A. and Singh, R.K. (2007). Cultural significance and diversities of ethnic foods of Northeast India. *Indian Journal of Traditional Knowledge* 6(1): 79-94.

Singh, N.L., Ramprasad Mishra, P.K., Shukla, S.K., Kumar, J. and Singh, R. (2010). Alcoholic fermentation techniques in early Indian tradition. *Indian Journal of History of Science* 45(2): 163-173.

Singh, P. and Chauhan, M. (2013). Influence of environmental factors on the growth of building deteriorating fungi: *Aspergillus flavus* and *Penicillium chrysogenum*. *International Journal of Pharmaceutical Sciences and Research* 4(1): 425.

Singh, N.K., Joshi, D.K. and Gupta, R.K. (2013a). Isolation of phytase producing bacteria and optimization of phytase production parameters. *Jundishapur Journal of Microbiology* 6(5): 1J.DOI: 10.5812/jjm.6419.

Singh, S. and Gupta, A. (2014). Comparative fermentation studies on amylase production by *Aspergillus flavus* TF-8 using Sal (*Shorea robusta*) deoiled cake as natural substrate: Characterization for potential application in detergency. *Industrial Crops and Products* 57: 158-165.

Singh, S., Singh, S., Bali, V., Sharma, L. and Mangla, J. (2014). Production of fungal amylases using cheap, readily available agriresidues, for potential application in textile industry. *BioMed research international* 2014: 9. doi.org/10.1155/2014/215748.

Singh, R., Kumar, M., Mittal, A. and Mehta, P.K. (2016). Microbial enzymes: industrial progress in 21st century. *3 Biotech* 6(2): 174. doi: 10.1007/s13205-016-0485-8.

Skory, C.D., Freer, S.N. and Bothast, R.J. (1997). Screening for ethanol-producing filamentous fungi. *Biotechnology Letters* 19: 203–206.

Slivinski, C.T., Machado, A.V.L., Iulek, J., Ayub, R.A. and Almeida, M.M.D. (2011). Biochemical characterisation of a glucoamylase from *Aspergillus niger* produced by solid-state fermentation. *Brazilian Archives of Biology and Technology* 54(3): 559-568.

Smid, E.J., Erkus, O., Spus, M., Wolkers-Rooijackers, J.C., Alexeeva, S. and Kleerebezem, M. (2014). Functional implications of the microbial community structure of undefined mesophilic starter cultures. *Microbial Cell Factories* 13: 52. doi.org/10.1186/1475-2859-13-S1-S2.

Soedarsono, J. (1972). Some notes on ragi tapé an inoculum for tapé fermentation. *MajalahIlmu Pertanian* 1: 235-241.

Song, S.H., Lee, C., Lee, S., Park, J.M., Lee, H.J., Bai, D.H., Yoon, S.S., Choi, J.B. and Park, Y.S. (2013). Analysis of microflora profile in Korean traditional nuruk. *Journal of Microbiology and Biotechnology* 23: 40-46.

Songulashvili, G., Elisashvili, V., Wasser, S.P., Nevo, E. and Hadar, Y. (2007). Basidiomycetes laccase and manganese peroxidase activity in submerged fermentation of food industry wastes. *Enzyme and Microbial Technology* 41(1-2): 57-61.

Souza, P.M.D. (2010). Application of microbial α-amylase in industry-A review. *Brazilian Journal of Microbiology* 41(4): 850-861.

Souza, P.M.D., Bittencourt, M.L.D.A., Caprara, C.C., Freitas, M.D., Almeida, R.P.C.D., Silveira, D., Fonseca, Y.M., Filho, E.X.F., Junior, A.P. and Magalhães, P.O. (2015). A biotechnology perspective of fungal proteases. *Brazilian Journal of Microbiology* 46(2): 337-346.

Souza, P.M., de Freitas, M.M., Cardoso, S.L., Pessoa, A., Guerra, E.N.S. and Magalhaes, P.O. (2017). Optimization and purification of L-asparaginase from fungi: *A systematic review. Critical Reviews in Oncology/hematology* 120: 194-202.

Spaho, N. (2017). Distillation techniques in the fruit spirits production. Distillation–Innovative Applications and Modeling. *InTech* 129-152.

Srivastava, N., Srivastava, M., Mishra, P.K., Gupta, V.K., Molina, G., Rodriguez-Couto, S., Manikanta, A. and Ramteke, P.W. (2018). Applications of fungal cellulases in biofuel production: advances and limitations. *Renewable and Sustainable Energy Reviews* 82:2379-2386.

Steinkraus, K.H. (1983). Lactic acid fermentation in the production of foods from vegetables, cereals and legumes. *Antonie van Leeuwenhoek* 49(3): 337-348.

Steinkraus, K.H. (1994). Nutritional significance of fermented foods. *Food Research International* 27(3): 259-267.

Steinkraus, K.H. (1996). *Handbook of Indigenous Fermented Foods.* 2nd ed. Marcel Dekker, New York.

Steinkraus, K.H. (1997). Classification of Fermented Foods: Worldwide Review of Household Fermentation Techniques. *Food Control* 8(5/6): 311-17.

Steinkraus, K.H. (2002). Fermentations in world food processing.*Comprehensive Reviews in Food Science and Food Safety 1*(1), 23-32.

Suganuma, T., Fujita, K. and Kitahara, K. (2007). Some distinguishable properties between acid-stable and neutral types of α-amylases from acid-producing koji. *Journal of Bioscience and Bioengineering* 104(5): 353-362.

Sunitha, V.H., Ramesha, A., Savitha, J. and Srinivas, C. (2012). Amylase production by endophytic fungi *Cylindrocephalum* sp. isolated from medicinal plant *Alpinia calcarata* (Haw.) Roscoe. *Brazilian Journal of Microbiology* 43: 1213-1221.

Suzuki, U. (1907). Uber ein Enzym" Phytase" das Anhydro-oxy-methylen-diphosphosaures paltet. *Bulletin of the College of Agriculture, Tokyo Imperial University* 7: 503-505.

Tang, Q., He, G., Huang, J., Wu, C., Jin, Y. and Zhou, R. (2019). Characterizing relationship of microbial diversity and metabolite in Sichuan *xiaoqu*. *Frontiers in Microbiology* 10: 696. doi.org/10.3389/fmicb.2019.00696.

Tamang, J.P. (2005). *Food Culture of Sikkim*. Sikkim Study Series volume IV. Information and Public Relations Department, Government of Sikkim, Gangtok, pp. 120.

Tamang, J.P. (2010a). Diversity of fermented beverages. In: *Fermented Foods and Beverages of the World* (Eds: Tamang, J.P. and Kailasapathy, K.), pp. 85-125. CRC Press, Taylor & Francis Group, New York.

Tamang, J.P. (2010b). *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values.* CRC Press, Taylor & Francis Group, New York.

Tamang, J.P. (2012a). Plant-Based Fermented Foods and Beverages of Asia. In: *Handbook of Plant-Based Fermented Food and Beverage Technology* (Eds. Hui, Y.H. and Özgül, E.), pp. 49–90. Second Edition, CRC Press, Taylor and Francis Group, New York.

Tamang, J.P. (2016). *Ethnic Fermented Foods and Alcoholic Beverages of Asia*. Springer, New Delhi.

Tamang, J.P. (2020). History and Culture of Indian Ethnic Fermented Foods and Beverages. In: *Ethnic Fermented Foods and Alcoholic Beverages of India: Science History and Culture* (Ed: Tamang, J.P.), pp. 1-40. Springer Nature, Singapore.

Tamang, J.P., Cotter, P., Endo, A., Han, N.S., Kort, R., Liu, S.Q., Mayo, B., Westerik, N. and Hutkins, R. (2020). Fermented foods in a global age: east meets west. *Comprehensive Reviews in Food Science and Food Safety* 19: 184-217. doi: 10.1111/1541-4337.12520.

Tamang, J.P. and Fleet, G.H. (2009). Yeasts diversity in fermented foods and beverages, In: *Yeasts Biotechnology: Diversity and Applications* (Eds: Satyanarayana, T. and Kunze, G.), pp. 169-198. Springer, New York.

Tamang, J.P., Holzapfel, W.H. and Watanabe, K. (2016a). Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology* 7:377. doi: 10.3389/fmicb.2016.00377.

Tamang, J.P., Shin, D.H., Jung, S.J. and Chae, S.W. (2016b). Functional properties of microorganisms in fermented foods. *Frontiers in Microbiology* 7:578. doi: 10.3389/fmicb.2016.00578.

Tamang, J.P., Thapa, N., Savitri. and Bhalla, T.C. (2016c). Ethnic fermented foods and beverages of India. In: *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (Ed. Tamang, J.P.), pp. 17-72. Springer, New Delhi.

Tamang, J.P. and Samuel, D. (2010). Dietary culture and antiquity of fermented foods and beverages. In*: Fermented Foods and Beverages of the World* (Eds: Tamang, J.P. and Kailasapathy, K), pp. 1–40. CRC Press, Taylor & Francis Group, New York.

Tamang, J.P., Thapa, N., Tamang, B., Rai, A. and Chettri, R. (2015). Microorganisms in fermented foods and beverages. In: *Health Benefits of Fermented Foods* (Ed: Tamang, J.P.), pp. 1-110. CRC Press, Taylor & Francis Group, New York.

Tamang J.P., Thapa, N., Dewan S., Tamang, B.M., Yonzan, H., Rai, A.K, Chettri, R., Chakrabarty, J. and Kharel, N. (2012). Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India. *Indian Journal of Traditional Knowledge* 11: 7-25.

Tamang, J.P., Dewan, S., Tamang, B., Rai, A., Schillinger, U. and Holzapfel, W.H. (2007). Lactic acid bacteria in hamei and marcha of North East India. *Indian Journal of Microbiology* 47(2): 119-125.

Tamang, J.P., Thapa, S., Tamang, N. and Rai, B. (1996). Indigenous fermented food beverages of Darjeeling hills and Sikkim: process and product characterization. *Journal of Hill Research* 9(2): 401-411.

Tamang, J.P. and Sarkar, P.K. (1995). Microflora of marcha: an amylolytic fermentation starter. *Microbios* 81:115– 122.

Tamang, J.P., Sarkar, P.K. and Hesseltine, C.W. (1988). Traditional fermented foods and beverages of Darjeeling and Sikkim - a review. *Journal of Science of Food and Agricultural* 44(4): 375–385.

Tamang, J.P. and Thapa, S. (2006). Fermentation dynamics during production of *bhaati jaanr,* a traditional fermented rice beverage of the Eastern Himalayas. *Food Biotechnology* 20 (3): 251-261.

Tanimura, W., Sanchez, P.C. and Kozaki, M. (1977). The fermented food in the Philippines *Tapuy* (rice wine). *Journal of Agricultural Science of the Tokyo University of Agriculture* 22(1): 118-134.

Tavano, O.L., Berenguer-Murcia, A., Secundo, F. and Fernandez-Lafuente, R. (2018). Biotechnological applications of proteases in food technology. *Comprehensive Reviews in Food Science and Food Safety* 17(2): 412-436.

Tedersoo, L., Sánchez-Ramírez, S., Koljalg, U., Bahram, M., Döring, M., Schigel, D., May, T., Ryberg, M. and Abarenkov, K. (2018). High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Diversity* 90(1): 135-159.

Teramoto, Y., Yoshida, S. and Ueda, S. (2002). Characteristics of a rice beer (zutho) and a yeast isolated from the fermented product in Nagaland, India. *World Journal of Microbiology and Biotechnology* 18(9): 813-816.

Terrone, C.C., de Freitas, C., Terrasan, C.R.F., de Almeida, A.F. and Carmona, E.C. (2018). Agroindustrial biomass for xylanase production by *Penicillium chrysogenum:* purification, biochemical properties and hydrolysis of hemicelluloses. *Electronic Journal of Biotechnology* 33: 39-45.

Thakur, N. and Bhalla, T.C. (2004). Characterization of some traditional fermented foods and beverages of Himachal Pradesh. *Indian Journal of Traditional Knowledge* 3(3): 325-335.

Thakur, N., Saris, P.E. and Bhalla, T.C. (2015). Microorganisms associated with amylolytic starters and traditional fermented alcoholic beverages of North Western Himalayas in India. *Food Bioscience* 11: 92-96.

Thanh, V.N. and Tuan, D.A. (2008). Microbial diversity of traditional Vietnamese alcohol fermentation starters (banh men) as determined by PCR-mediated DGGE. *International Journal of Food Microbiology* 128(2): 268-273.

Thapa, N. and Tamang, J.P. (2020). Ethnic Fermented Foods and Beverages of Sikkim and Darjeeling Hills (Gorkhaland Territorial Administration). In: *Ethnic Fermented Foods and Alcoholic Beverages of India: Science History and Culture* (Ed: Tamang, J.P.), pp. 479-537. Springer Nature, Singapore.

Thapa, S. and Tamang, J.P. (2004). Product characterization of kodo ko jaanr: fermented finger millet beverage of the Himalayas. *Food Microbiology* 21: 617-622.

Thongekkae, J. and Kongsanthia, J. (2016). Screening and Identification of Cellulase Producing Yeast from Rongkho Forest, Ubon Ratchathani University. *Bioengineering and Bioscience* 4(3): 29-33.

Tiwari, S.C. and Mahanta, D. (2007). Ethnological observations on fermented food products of certain tribes of Arunachal Pradesh. *Indian Journal of Traditional Knowledge* 6(1): 106-110.

Tomšovský, M. and Homolka, L. (2003). Laccase and other ligninolytic enzyme activities of selected strains of *Trametes* spp. from different localities and substrates. *Folia Microbiologica* 48(3): 413.

Tsang, C.C., Tang, J.Y., Lau, S.K. and Woo, P.C. (2018). Taxonomy and evolution of *Aspergillus, Penicillium* and *Talaromyces* in the omics era–Past, present and future. *Computational and Structural Biotechnology Journal*. 16 197-210.

Tsuyoshi, N., Fudou, R., Yamanaka, S., Kozaki, M., Tamang, N., Thapa, S. and Tamang, J.P. (1995). Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation. *International Journal of Food Microbiology* 99(2): 135-146.

Tsuyoshi, N., Fudou, R., Yamanaka, S., Kozaki, M., Tamang, N., Thapa, S. and Tamang, J.P. (2005). Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation. *International Journal of Food Microbiology* 99(2): 135-146.

Uchida, M., Hideshima, N. and Araki, T. (2019). Development of *koji* by culturing *Aspergillus oryzae* on nori (*Pyropia yezoensis*). *Journal of Bioscience and Bioengineering* 127(2): 183-189.

Uchimura, T, Kojima, Y. and Kozaki, M. (1990) Studies on the main saccharifying microorganism in the Chinese starter of Bhutan "Chang poo". *Journal of Brewing Society of Japan* 85(12): 881-887.

Umesha, S., Manukumar, H.M. and Raghava, S. (2016). A rapid method for isolation of genomic DNA from food-borne fungal pathogens. *3 Biotech* 6(123). doi:10.1007/s13205-016-0436-4.

Unterseher, M., Schnittler, M., Dormann, C. and Sickert, A. (2008). Application of species richness estimators for the assessment of fungal diversity. *FEMS Microbiology Letters* 282(2): 205–213.

Vaishali, P. and Bhupendra, N. (2017). A rapid and efficient dye based plate assay technique for screening of l-asparaginase producing fungal strains. *Journal of. Microbial and. Biochemical Technology* 9: 162-168.

Van Laarhoven, K.A., Huinink, H.P., Segers, F.J., Dijksterhuis, J. and Adan, O.C. (2015). Separate effects of moisture content and water activity on the hyphal

extension of *Penicillium rubens* on porous media. *Environmental Microbiology* 17(12): 5089-5099.

Vashishth, A., Ram, S. and Beniwal, V. (2017). Cereal phytases and their importance in improvement of micronutrients bioavailability. 3 *Biotech* 7(1): 42. doi.org/10.1007/s13205-017-0698-5.

Verma, P. and Verma, R.K. (2016). Cellulase activity of soil fungi (*Aspergillus, Fusarium, Penicillium, Trichoderma*) isolated from rhizosphere region of iron ore mine overburden soil. *International Journal of Basic and Applied Biology* 3(2): 115-120.

Verni, M., Rizzello, C.G. and Coda, R. (2019). Fermentation biotechnology applied to cereal industry by-products: nutritional and functional insights. *Frontiers in Nutrition* 6.doi.org/10.3389/fnut.2019.00042.

Villena, G.K. and Gutiérrez-Correa, M. (2006). Production of cellulase by *Aspergillus niger* biofilms developed on polyester cloth. *Letters in Applied Microbiology* 43(3): 262-268.

Vogel, R.F., Hammes, W.P., Habermeyer, M., Engel, K.H., Knorr, D. and Eisenbrand, G. (2011). Microbial food cultures–opinion of the Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG). *Molecular Nutrition & Food Research* 55(4): 654-662.

Wadia, T. and Jain, K.S. (2017). Isolation, screening and identification of lipase producing fungi from oil contaminated soil from Shani Mandir Ujjain. *International Journal of Current Microbiology and Applied Sciences* 6(7): 1872-1878.

Wagner, L., Stielow, J.B., de Hoog, G.S., Bensch, K., Schwartze, V.U., Voigt, K., Alastruey-Izquierdo, A., Kurzai, O. and Walther, G. (2019). A new species concept for the clinically relevant *Mucor circinelloides* complex. *Persoonia* 44: 67-97.

Walia, A., Guleria, S., Mehta, P., Chauhan, A. and Parkash, J. (2017). Microbial xylanases and their industrial application in pulp and paper bio-bleaching: a review. *3 Biotech* 7(1): 11. doi.org/10.1007/s13205-016-0584-6.

Walker, G.M. and White, N.A. (2017). Introduction to fungal physiology. Fungi: biology and applications, In: *Fungi: biology and applications* (Ed: Kavangh, K.) pp. 1-35 John Wiley and Son. doi.org/10.1002/9781119374312.ch1.

Walker, G.M. and Stewart, G.G. (2016). *Saccharomyces cerevisiae* in the production of fermented beverages. *Beverages* 2: 30.doi:10.3390/beverages2040030.

Walsh, A.M., Crispie, F., Kilcawley, K., O'Sullivan, O., O'Sullivan, M.G., Claesson, M.J. and Cotter, P.D. (2016). Microbial succession and flavor production in the fermented dairy beverage kefir. *Msystems* 1(5): e00052-16. DOI: 10.1128/mSystems.00052-16.

Wanderley, K.A., Oliveira, I.S. and Cruz, R. (2017). Antimicrobial and enzymatic activity of anemophilous fungi of a public university in Brazil. *Annals of the Brazilian Academy of Sciences* 89(3): 2327-2340.

Wang, M.Y., Zhao, Q.S., Su, C. and Yang, J.G. (2019). Analysis of the microbial community structure during brewing of Sichuan Xiaoqu Baijiu. *Journal of the American Society of Brewing Chemists* 77(3): 210-219.

Wang, Q., Garrity, G.M., Tiedje, J.M. and Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73(16): 5261-5267.

Watanabe, T. (2010). *Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species*. CRC press, Boca Raton, Fla.

Wei, H., Wang, W., Yarbrough, J.M., Baker, J.O., Laurens, L. and Van Wychen, S. (2013). Genomic, proteomic, and biochemical analyses of oleaginous *Mucor circinelloides*: evaluating its capability in utilizing cellulolytic substrates for lipid production. *Plos One* 8(9): e71068. doi.org/10.1371/journal.pone.0071068.

Whittaker, R.H. (1969). New concepts of kingdoms of organisms. *Science* 163(3863): 150-160.

Wu, B., Hussain, M., Zhang, W., Stadler, M., Liu, X. and Xiang, M. (2019). Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. *Mycology* 10(3): 127-140.

Wyss, M., Brugger, R., Kronenberger, A., Rémy, R., Fimbel, R., Oesterhelt, G., Lehman, M. and van Loon, A.P. (1999). Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. *Applied and Environmental Microbiology* 65(2): 367-373.

Xing, J.H., Sun, Y.F., Han, Y.L., Cui, B.K. and Dai, Y.C. (2018). Morphological and molecular identification of two new Ganoderma species on *Casuarina equisetifolia* from China. *MycoKeys* 34: 93-108.

Xu, J. (2016). Fungal DNA barcoding. *Genome* 59(11): 913-932.

Xu, Q.S., Yan, Y.S. and Feng, J.X. (2016). Efficient hydrolysis of raw starch and ethanol fermentation: a novel raw starch-digesting glucoamylase from Penicillium oxalicum. *Biotechnology for Biofuels* 9(216). doi.org/10.1186/s13068-016-0636-5.

Xu, Y., Sun, B., Fan, G., Teng, C., Xiong, K., Zhu, Y., Li, J. and Li, X. (2017). The brewing process and microbial diversity of strong flavour Chinese spirits: a review. *Journal of the Institute of Brewing* 123(1): 5-12.

Yaicin, H.T. and Corbaci, C. (2013). Isolation and Characterization of Amylase Producing Yeasts and Improvement of Amylase Production. *Turkish Journal of Biochemistry* 38(1):101-108.

Yamada, H., Adachi, O., Watanabe, M. and Sato, N. (1968). Studies on fungal tannase: part I. formation, purification and catalytic properties of tannase of *Aspergillus flavus. Agricultural and Biological Chemistry* 32(9): 1070-1078.

Yamamoto, S. (2016). Ethnic fermented foods and beverages of Cambodia. In: *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (Ed. Tamang, J.P.), pp. 237-262. Springer, New Delhi.

Yang, S., Lee, J., Kwak, J., Kim, K., Seo, M. and Lee, Y.W. (2011). Fungi associated with the traditional starter cultures used for rice wine in Korea. *Journal of Korean Society of Applied Biology and Chemistry* 54(6): 933-943.

Yuan, L., Wang, W., Pei, Y. and Lu, F. (2012). Screening and identification of cellulase-producing strain of *Fusarium oxysporum. Procedia Environmental Sciences* 12, 1213-1219.

Zakipour-Molkabadi, E., Hamidi-Esfahani, Z., Sahari, M.A. and Azizi, M.H. (2013). A new native source of tannase producer, *Penicillium* sp. EZ-ZH190: Characterization of the enzyme. *Iranian Journal of Biotechnology* 11(4): 244-250.

Zhang, X.Z. and Zhang, Y.H.P. (2013). Cellulases: characteristics, sources, production, and applications. *Bioprocessing technologies in biorefinery for sustainable production of fuels. Chemicals, and Polymers* 1: 131-146.

Zhang, Y., Fraatz, M.A., Müller, J., Schmitz, H.J., Birk, F., Schrenk, D. and Zorn, H. (2015). Aroma characterization and safety assessment of a beverage fermented by *Trametes versicolor*. *Journal of Agricultural and Food Chemistry* 63(31): 6915-6921.

Zheng, X.W., Tabrizi, M.R., Nout, M.R. and Han, B.Z. (2011). Daqu—a traditional Chinese liquor fermentation starter. *Journal of the Institute of Brewing* 117(1): 82-90.

Zheng, X.W., Yan, Z., Han, B.Z., Zwietering, M.H., Samson, R.A., Boekhout, T. and Nout, M.R. (2012). Complex microbiota of a Chinese "Fen" liquor fermentation starter (Fen-Daqu), revealed by culture-dependent and culture-independent methods. *Food Microbiology* 31(2): 293-300.

Zheng, X.W., Yan, Z., Nout, M.R., Boekhout, T., Han, B.Z., Zwietering, M.H. and Smid, E.J. (2015). Characterization of the microbial community in different types of Daqu samples as revealed by 16S rRNA and 26S rRNA gene clone libraries. *World Journal of Microbiology and Biotechnology* 31(1): 199-208.

Zhou, B., Ma, C., Wang, H. and Xia, T. (2018). Biodegradation of caffeine by whole cells of tea-derived fungi *Aspergillus sydowii*, *Aspergillus niger* and optimization for caffeine degradation. *BMC Microbiology* 18(1): 53. doi: 10.1186/s12866-018-1194-8.

Zhou, X. and Li, Y. (2015). Atlas of Oral Microbiology: From Healthy Microflora to Disease. Academic Press. 15-40. doi.org/10.1016/B978-0-12-802234-4.00002-1.

Zou, W., Zhao, C. and Luo, H. (2018). Diversity and function of microbial community in chinese strong-flavor baijiu ecosystem: a review. *Frontiers in Microbiology* 9:671. doi:10.3389/fmicb.2018.00671.

Zulkifli, N.A. and Zakaria, L. (2017). Morphological and molecular diversity of *Aspergillus* from corn grain used as livestock feed. *Hayati Journal of Biosciences* 24(1): 26-34.

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QUALIFICATION

Degree	University	Month and year of joining	Month and year of passing	Marks (%)
MPhil (Microbiology)	Sikkim University	06/2012	12/2014	66
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BSc. (Microbiology)	BVU, Pune	06/2006	05/2009	64

Research Experience: Expertise in phenotypic characterization of **Fungi** and bacteria, molecular techniques like Genomic DNA isolation, PCR analysis, Phylogeny Tree Constructions. Fungal diversity using Culture-independent method (High-throughput Amplicon Sequencing).

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1) International Conference on "Ethnic Fermented Foods and Beverages: Microbiology and Health Benefits" at Sikkim University, Gangtok: 20-21 Nov, 2015
2) 6th AIST International Imaging Workshop & DAILAB PIKNIKH Series XXXII held at Biomedical Research Institute, AIST, Tsukuba Science City, Japan supported by at DAICENTER, AIST & JST and DBT from January 20-27, 2019

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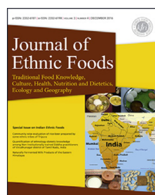
Workshop/Conferences Attended

1) Hands on training on fungal Identification at National Fungal Culture Collection of India (NFCCI) Agharkar Research Institute, Gopal Ganesh Agharkar Road, Pune 411004, Maharashtra, India. (January to February 2018).
2) The 6th AIST international Imaging workshop & DAILAB PIKNIKH series XXXII, organized by AIST, Tsukuba, Japan on January 20-27, 2019.

Publications

1. Sha, S.P., **Anupma, A.**, Pradhan, P., Prasad, G.S. and Tamang, J.P. (2016). Identification of yeasts by polymerase-chain-reaction-mediated denaturing gradient gel electrophoresis in *marcha,* an ethnic amylolytic starter of India. *Journal of Ethnic Foods* 3: 292-296.
2. Sha, S.P., Jani, K., Sharma, **A., Anupma**, A., Pradhan, P., Shouche, Y. and Tamang, J.P. (2017). Analysis of bacterial and fungal communities in *Marcha* and *Thiat*, traditionally prepared amylolytic starters of India. *Scientific Reports* 7: 10967. doi: 10.1038/s41598-017-11609-y. (Impact Factor: 4.525).
3. **Anupma, A**., Pradhan, P., Sha, S.P. and Tamang, J.P. (2018). Traditional skill of ethnic people of the Eastern Himalayas for preservation of microbiota in form of dry amylolytic starters for production of alcoholic beverages. *Indian Journal of Traditional Knowledge* 17: 184-190. (Impact Factor: 0.920).
4. **Anupma, A**. and Tamang, J.P. (2020). Diversity of filamentous fungi isolated from some amylase and alcohol-producing starters of India. *Frontiers in Microbiology* 11:905.doi: 10.3389/fmicb.2020.00905 (Impact Factor: 4.259).

J Ethn Foods 3 (2016) 292–296



Contents lists available at ScienceDirect

Journal of Ethnic Foods

journal homepage: http://journalofethnicfoods.net

Original article

Identification of yeasts by polymerase-chain-reaction-mediated denaturing gradient gel electrophoresis in *marcha*, an ethnic amylolytic starter of India

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ARTICLE INFO

Article history:
Received 15 October 2016
Received in revised form
28 November 2016
Accepted 28 November 2016
Available online 2 December 2016

Keywords:
amylolytic starter
denaturing gradient gel electrophoresis
marcha
polymerase chain reaction
Sikkim
Wickerhamomyces anomalus

ABSTRACT

Background: *Marcha* is an ethnic amylolytic starter that is used to ferment boiled cereals to produce alcoholic drinks, commonly called *jaanr*, in the Himalayan Regions of Sikkim and Darjeeling of India.
Methods: The aim of this study was to investigate yeast flora of *marcha* collected from Sikkim in India by phenotypic characterization and polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE).
Results: The average load of yeast in *marcha* was 6.0×10^8 colony-forming units/g. The phenotypic characterization of yeast isolates from *marcha* showed the presence of *Candida*, *Pichia*, *Torulospora*, *Schizosaccharomyces, Kluveromyces, Issatchenki*, and *Saccharomycopsis*. The PCR-DGGE bands showed the dominance of *Wickerhamomyces anomalus* (72%) and *Pichia anomalus* (28%) in *marcha*. *W. anomalus* was reported for the first time from *marcha* using PCR-mediated DGGE.
Conclusion: This is the first report on the yeast community associated with *marcha* analyzed by PCR-mediated DGGE.
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1. Introduction

The traditional way of preparation of amylolytic starters is a unique technology of preservation of native microorganisms, consisting of consortia of amylolytic and alcohol-producing yeasts, molds, and some lactic-acid bacteria, with rice or wheat as the base in the form of dry, flattened, or round balls, for alcoholic beverage production in Asia [1]. Amylolytic starters in Asia have different vernacular names such as *marcha* in India, Nepal, and Bhutan, *hamei*, *humao*, and *phab* in India [2–4], *mana* and *manapu* in Nepal [5], *men* in Vietnam [6], *bubod* in the Philippines [7], *chiu/chu* in China and Taiwan [8], *loogpang* in Thailand [9], *ragi* in Indonesia [10], and *nuruk* in Korea [8]. *Marcha* is a nonfood starter culture uses for production of various ethnic alcoholic beverages in the Darjeeling Hills and Sikkim in India, Nepal, and Bhutan [3]. It is a dry, round-to-flattened, creamy to dusty white, solid ball-like starter (Fig. 1). During its preparation, soaked glutinous rice is crushed in a foot-driven heavy wooden mortar, with the addition of the roots of *Plumbago zeylanica* L., leaves of *Buddleja asiatica* Lour, flowers of *Vernonia cinerea* (L.) ginger, red dry chili, and 1% of previously prepared powdered *marcha* for back-sloping fermentation [2]. The mixed dough is kneaded into round or flat cakes of different sizes and shapes that are placed individually on a platform suspended below the bamboo-made ceiling above the earthen kitchen, bedded with fresh fronds of fern *Glaphylopteriolopsis erubescens* (Wall ex Hook.) Ching, and covered with dry fronds of fern and jute bags and are then left to ferment for 1–3 days. Finally, cakes of *marcha* are sun dried for 2–3 days and stored in a dry place at room temperature for > 1 year. Application of polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE), a culture-independent method, is widely applied to study microbial diversity [11–13]. Some species of yeasts such as *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Saccharomycopsis*, *Schizosaccharomyces*, *Torulopsis*, and *Zygosaccharomyces* were previously reported from samples of *marcha* using culture-dependent approaches [7,14,15]. However, a culture-independent method using PCR-DGGE has not

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http://dx.doi.org/10.1016/j.jef.2016.11.009
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Fig. 1. *Marcha*, amylolytic starter of Sikkim in India.

been applied yet in profiling of yeast flora in *marcha*. The present study aimed to profile yeast flora directly from *marcha* samples using PCR-mediated DGGE.

2. Materials and methods

2.1. Sample collection

Ten samples of dry *marcha* were collected from the local market or villages of Sikkim in presterile polythene bags, and were stored in a desiccator at room temperature until analysis.

2.2. Culture-dependent analysis

2.2.1. Isolation of microorganisms

Ten grams of powdered *marcha* was mixed in 90 mL physiological saline (0.85%) and homogenized in a Stomacher Lab-Blender 400 (Seward, Worthing, UK) for 1 minute. Serial dilutions were prepared in sterile diluent and mixed with the molten media and poured into plates. Plates of yeast extract–malt extract agar (M424; HiMedia, Mumbai, India) for enumeration of yeasts were incubated at 30°C for 48 hours. Yeast isolates were purified and preserved at −20°C in yeast extract–malt extract broth (M425; HiMedia) mixed with 20% (v/v) glycerol.

2.2.2. Phenotypic characterization

Cell morphology of yeast isolates was determined using a phase contrast microscope (CH3-BH-PC; Olympus, Tokyo, Japan). Yeast cultures have been characterized on the basis of mycelium type, ascospore type, nitrate reduction, growth at 37°C and 45°C, sugar fermentation, and sugar assimilation following the methods of Kurtzman et al [16].

2.3. Culture-independent analysis

2.3.1. DNA extraction from sample

Ten grams of powdered *marcha* was homogenized in 90 mL of 0.85% w/v sterile physiological saline, and subsequently filtered. The resulting filtered solutions were centrifuged at 14,000 *g* for 10 minutes at 4°C, and pellets were subjected to DNA extraction using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) after glass bead (0.2–0.5 mm diameter; Sigma-Aldrich, Roth, Germany) beating to rupture the cell walls. The yield and quality of DNA were detected through agarose gel electrophoresis (1.0%), which was stained with ethidium bromide solution.

2.3.2. PCR amplification and DGGE analysis

PCR-DGGE analysis was performed as described previously [17]. Coated PCR with primers sets ITS1-F, ITS4, ITS2, and ITS1F-GC was used to amplify yeast ITS region [18]. A 40-base (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3′) attached to the 5′ end of the ITS1-F primer was used to stabilize the melting behavior of the DNA fragments during DGGE analysis [19]. The first round of PCRs was carried out in a Mastercycler (Applied Biosystems, Foster City, CA, USA) using 25-μL reaction volumes containing: 1 μL DNA template, 0.25 μL each primer (10μM), 12.5 μL 2× Go Taq Master Mix (Promega), and 11 μL nuclease-free water. PCR cycle was programed as follows: 94°C for 4 minutes followed by 10 cycles of 94°C for 1 minutes, lowering the annealing temperature from 65°C to 55°C in 1°C steps for each cycle for 1 minute, 72°C for 1 minute, and finally 25 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and followed by a final extension at 72°C for 7 minutes. The second PCRs were carried out in a Mastercycler (Applied Biosystems) using 50-μL reaction volumes containing: 1 μL first PCR production, 0.5 μL each primer (10μM), 25 μL 2× Go Taq Master Mix (Promega) and 23 μL nuclease-free water. Cycling parameters were the same as for the first round of PCR. All amplified products were analyzed by electrophoresis in 1.2% (w/v) agarose gel, stained with ethidium bromide, and visualized under UV light. DGGE analysis was carried out using the PCR products in an universal mutation detection system (DGGEK-1001-220; CBS Scientific, Del Mar, CA, USA) following the procedure described by El Sheikha et al [20]. Samples containing approximately equal amounts of PCR amplicons (30 μL) were loaded into 8% (w/v) polyacrylamide gels (acrylamide/N, N0-methylene bisacrylamide, 37.5/1; Promega) in 1× TAE buffer (40mM Tris–HCl pH 7.4, 20mM sodium acetate, 1.0mM Na_2-EDTA). Electrophoresis was performed at 60°C in a denaturing gradient ranging from 40% to 60% [100% corresponded to 7M urea and 40% (v/v) formamide; Promega], at 20 V for 10 minutes and then at 130 V for 4.5 hours, and the gels were stained for 30 minutes with ethidium bromide and then photographed on a gel documentation unit (GelDoc 1000; Bio-Rad, Hercules, CA, USA).

2.3.3. Identification of bands

Individual DGGE bands were excised, resuspended in 20 μL sterile Tris–EDTA buffer, and stored at 4°C overnight. An aliquot of supernatant was used as a DNA template for PCR reamplification as described above, and electrophoresed with DGGE. Band excision, PCR, and DGGE were repeated until a single band was present. PCR products generated from DGGE bands were amplified with primers ITS2 and ITS-1f (without the GC clamp) for sequencing using DNA sequencer (Applied Biosystems). Sequences of major bands obtained from the DGGE gel fragments were compared with the GenBank database using the web-based nucleotide–nucleotide BLAST search engine hosted by the National Center for Biotechnology Information (Bethesda, MD, USA) for identification (http://www.ncbi.nlm.nih.gov) [21].

2.3.4. Phylogenetic analysis

The BLAST program was used for comparing DNA databases for sequence similarities available on the EzTaxon server (http://eztaxon-e.ezbiocloud.net/). Molecular evolutionary genetics analysis software (MEGA version 6, US National Library of Medicine, National Institution of Health) was used for phylogenetic analyses [22].

Table 1
Characterization of yeasts isolates from *marcha*.

Representative strains*	No. of grouped strains	Pellicle formation	Nitrate reduction	Sugar fermentation†									Tentative genera
				Cellobiose	Arabinose	Ribose	Mannose	Raffinose	Aesculin	Galactose	Trehalodse	Xylose	
GM:Y12	2	+	−	−	−	+	+	+	+	+	−	+	*Saccharomyces*
GM:Y21	2	+	−	−	−	+	1/1	+	+	+	−	−	*Saccharomyces*
GM:Y50	3	+	−	2/1	−	+	+	+	+	2/1	+	+	*Saccharomyces*
GM:Y34	3	−	+	+	+	+	+	+	+	+	−	+	*Pichia*
GM:Y43	2	−	+	+	+	+	+	+	+	+	−	+	*Pichia*
GM:Y7	3	−	−	+	−	−	−	−	−	−	−	2/1	*Candida*
GM:Y37	2	−	−	+	−	−	−	−	−	−	−	+	*Candida*
GM:Y4	2	+	−	−	−	+	+	−	+	+	−	−	*Issatchenkia*
GM:Y36	2	+	−	−	−	+	+	−	+	+	−	−	*Issatchenkia*
GM:Y10	2	+	−	−	−	+	+	−	+	+	−	−	*Issatchenkia*
GM:Y29	4	+	−	+	+	3/1	+	+	+	+	+	−	*Kluveromyces*
GM:46	3	+	−	+	+	2/1	+	+	+	+	+	−	*Kluveromyces*
GM:Y5	3	+	−	+	+	+	+	+	+	+	+	−	*Schizosaccharomyces*
GM:49	2	+	−	+	+	+	+	+	+	+	+	−	*Schizosaccharomyces*
GM:Y15	3	+	−	+	+	+	+	+	+	+	+	−	*Schizosaccharomyces*
GM:Y22	2	+	−	+	1/1	+	+	+	+	+	+	+	*Saccharomycopsis*
GM:Y41	2	+	−	+	1/1	+	+	+	+	+	+	+	*Saccharomycopsis*
GM:Y1	4	+	−	−	+	+	3/1	+	+	+	+	+	*Torulospora*
GM:Y18	4	+	−	−	+	+	3/1	+	+	+	+	+	*Torulospora*

+, all strains positive; −, all strains negative; (../..), number of positive/negative strains.

* All yeast cells were oval to circular, colonies of all strains were smooth and creamy white, showed pseudo mycelia. All strains grew well at 37°C, but not at 45°C, except *Kluveromyces* grew at 45°C.

† All strains fermented sucrose, glucose, fructose, galactose, maltose, and no strains fermented lactose, except *Kluveromyces*, and all strains assimilated sugars except lactose.

3. Results

3.1. Phenotypic characterization of yeasts

The average load of yeast in *marcha* was calculated as 6.0×10^8 colony-forming units/g (data not shown). A total of 50 yeasts isolates were isolated from 10 samples of *marcha*. Characterization of yeasts isolated from different *marcha* samples of Sikkim, which were phenotypically identified on the basis of colony morphology, cell morphology sugar fermentation, and sugar assimilation tests (Table 1). Out of 50 isolates, 19 representative isolates were grouped based on colony appearance, cell shape, type of mycelia and ascospores, pellicle formation, nitrate reduction, and growth at 37°C and 45°C (Table 1). All 50 isolates were tested for sugar fermentation and sugar assimilation for identification up to genus level (Table 1). Tentatively the following yeast genera were identified: *Candida* (18%), *Pichia* (14%), *Torulospora* (14%), *Schizosaccharomyces* (16%), *Kluveromyces* (10%), *Issatchenki* (20%), and *Saccharomycopsis* (8%).

3.2. PCR-DGGE analysis

DNA was directly extracted from *marcha* samples. The results of PCR-DGGE analysis showed the diversity of yeast (Fig. 2). Five bands were identified as *Wickerhamomyces anamolus* and two bands as *Pichia anamolus*. Detected in eight of the 10 samples, *W. anamolus* (DGGE bands MY1, MY3, MY4, MY7, and MY8) was found to be the most abundant yeast species. *P. anamolus* (DGGE bands MY5 and MY6) was detected frequently in *marcha* (Fig. 2). Nevertheless, it is worthwhile to note that some yeast species were only detected by DGGE in some samples, for example, *W. anamolus* was found in *marcha* samples with an intense DGGE band. By contrast, *P. anamolus* was detected both by culture independent (PCR-DGGE) as well as culture-dependent techniques (conventional microbiological method). However, we were not able to identify the minor bands (MY9–MY13) since they could not be excised from the gels due to their low intensity.

The selected seven isolates were identified by partial 18S rRNA gene sequencing and were compared to the EzTaxon server database for their phylogenetic relationship using MEGA 6.06 version software (Fig. 3). Five of the isolates were identified as *W. anamolus* (MY1, MY3, MY4, MY7, and MY8) and two as *P. anamalus* (MY5, MY6). Identification based on BLAST comparison in GenBank of the bands obtained by PCR-DGGE gel using universal primers NL1/LS2 is shown in Table 2.

4. Discussion

The diversity of yeasts associated with amylolytic starters may be closely related to the raw material used and the regional climate

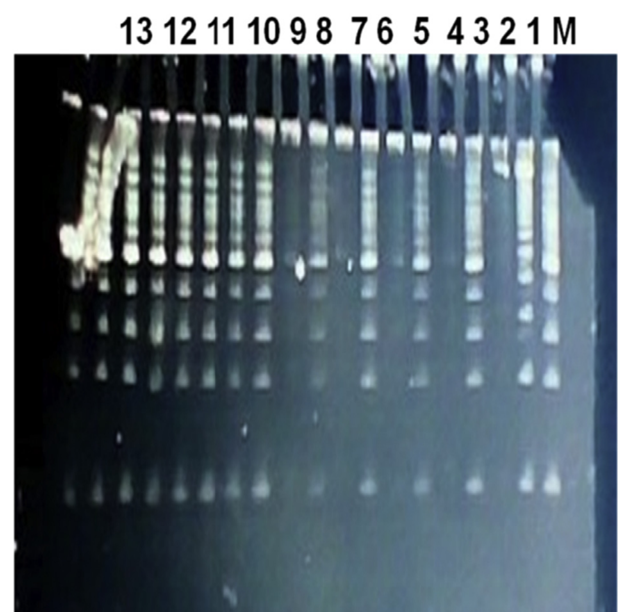


Fig. 2. Denaturing gradient gel electrophoresis profile of bands. 1, *Wickerhamomyces anomalus*; 2, 9, 10, and 11–13 unidentified; 3, *W. anomalus*; 4, *W. anomalus*; 5, *Pichia anomalus*; 6, *P. anomalus*; 7, *W. anomalus*; 8, *W. anomalus*.

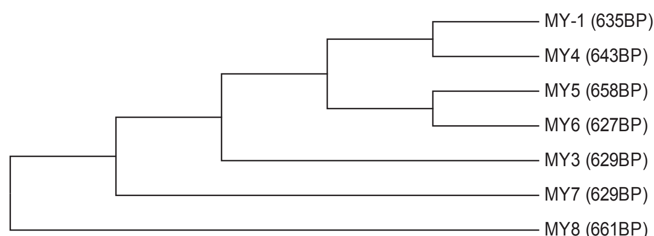


Fig. 3. Phylogenetic tree of yeasts retrieved from bands (MY1−MY8) in denaturing gradient gel electrophoresis profile by neighbor-joining distance tree constructed by MEGA 6 for the seven yeast isolates with the phylogenetic neighbors obtained from EzTaxon server.

Table 2
Identification based on BLAST comparison in GenBank of the bands obtained by polymerase chain reaction−denaturing gradient gel electrophoresis using universal primers NL1/LS2.

Band	Closest relative species	Accession No.	% identity
MY1	*Wickerhamomyces anomalus*	KT175181.1	99
MY3	*W. anomalus*	KT175201.1	99
MY4	*W. anomalus*	G0280811.1	99
MY5	*Pichia anomalus*	E0798697.1	98
MY6	*P. anomalus*	AY349435.1	99
MY7	*W. anomalus*	KT175181.1	99
MY8	*W. anomalus*	KT175181.1	99

where they are produced [23]. *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae*, *W. anomala*, *Pichia guilliermondii*, and *Candida* sp. are the most common yeasts present in rice-based starters of Asia [24−26]. It is interesting to find that yeast species (*W. anomalus*) could not be detected by conventional media but has been detected by PCR-DGGE analysis. *W. anomalus* has been reported in *hong-qu* and *yao-qu*, traditional amylolytic starters of China [27,28] and *banh men*, a traditional Vietnamese starter [29]. However, we noticed that *P. anomala*, *Issatchenkia*, *S. cerevisiae*, *Torulospora*, *Kluveromyces*, *Candida musae*, *S. fibuligera*, and *Saccharomycopsis* spp., which were detected in *marcha* through culture-dependent methods, were not detected in *marcha* in PCR-mediated DGGE assay. This discrepancy may have been due to the selective amplification, migration of PCR products from different species, and efficiencies of genomic DNA extraction kits for different species [30,31]. Such discrepancies between culturing and DGGE results have also been reported earlier [11,32]. Therefore, it is suggested that the DGGE technique be supplemented with culture-dependent methods, and their combination seems the best strategy to have a complete overview of yeast ecology of traditional alcoholic starters. *W. anomalus*, probably nonculturable yeast, was reported for the first time from *marcha* using PCR-mediated DGGE technique.

This is believed to be the first report on the yeast community associated with *marcha* of India analyzed by PCR-mediated DGGE. The results may enrich our knowledge of nonculturable native microorganisms that may be present in the traditionally prepared starters of Asia.

Conflict of interest

There is no conflict of interest.

Acknowledgments

The authors are grateful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for DBT-Twinning project to J.P.T. and G.S.P.

References

[1] Tamang JP. Diversity of fermented beverages. In: Tamang JP and Kailasapathy K, editors. Fermented foods and beverages of the world. Boca Raton: CRC Press, Taylor and Francis; 2010. p. 85−125.
[2] Tamang JP, Thapa S, Tamang N and Rai B. Indigenous fermented food beverages of Darjeeling hills and Sikkim: process and product characterization. J Hill Res 1996;9:401−11.
[3] Tamang JP. Fermented milks. In: Tamang JP, editor. Himalayan fermented foods: microbiology, nutrition, and ethnic values. Boca Raton: CRC Press, Taylor and Francis; 2010. p. 95−109.
[4] Tamang JP, Tamang N, Thapa S, Dewan S, Tamang BM, Yonzan H, Rai AK, Chettri R, Chakrabarty J and Kharel N. Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India. Indian J Traditional Know 2012;11:7−25.
[5] Nikkuni S, Karki TB, Terao T and Suzuki C. Microflora of mana, a Nepalese rice koji. J Fermen Bioeng 1996;81:168−70.
[6] Dung NTP, Rombouts FM and Nout MJR. Characteristics of some traditional Vietnamese starch-based rice wine fermentation starters (men). LWT-Food Sci Technol 2007;40:130−5.
[7] Hesseltine CW and Kurtzman CP. Yeasts in amylolytic food starters. Annls Inst Biol Uni Nac Antón México Ser Bot 1990;60:1−7.
[8] Steinkraus KH. Handbook of indigenous fermented food. 2nd ed. New York: Marcel Dekker, Inc.; 1996.
[9] Vachanavinich K, Kim WJ and Park YI. Microbial study on krachae, Thai rice wine. In: Lee CH, Adler-Nissen J and Bärwald G, editors. Lactic acid fermentation of non-alcoholic dairy food and beverages. Seoul: Ham Lim Won; 1994. p. 233−46.
[10] Uchimura T, Okada S and Kozaki M. Identification of lactic acid bacteria isolated from Indonesian Chinese starter, "ragi". Microorganisms in Chinese starters from Asia (Part 4). J Brew Soc Japan 1991;86:55−61.
[11] Ercolini D, Mauriello G, Blaiotta G, Moschetti G and Coppola S. PCR-DGGE fingerprints of microbial succession during a manufacture of traditional water buffalo mozzarella cheese. J Appl Microbiol 2004;96:263−70.
[12] Nielsen DS, Teniola OD, Ban-Koffi L, Owusu M, Andersson TS and Holzapfel WH. The microbiology of Ghanaian cocoa fermentations analysed using culture dependent and culture-independent methods. Int J Food Microbiol 2007;114:168−86.
[13] Tamang JP, Watanabe K and Holzapfel WH. Review: diversity of microorganisms in global fermented foods and beverages. Frontiers Microbiol 2016;7: 377. http://dx.doi.org/10.3389/fmicb.2016.00377.
[14] Tamang JP and Sarkar PK. Microflora of murcha: an amylolytic fermentation starter. Microbios 1995;81:115−22.
[15] Tsuyoshi N, Fudou R, Yamanaka S, Kozaki M, Tamang N, Thapa S and Tamang JP. Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation. Int J Food Microbiol 2005;99: 135−46.
[16] Kurtzman CP, Fell JW and Boekhout T. The yeasts: a taxonomic study. 5th ed. London: Elsevier; 2011.
[17] Cocolin L, Bisson LF and Mills DA. Direct profiling of the yeast dynamics in wine fermentations. FEMS Microbiol Lett 2000;189:81−7.
[18] Gardes M and Bruns TD. ITS primers with enhanced specificity for basidiomycetes −application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113−8.
[19] Sheffield VC, Cox DR, Lerman LS and Myers RM. Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc Natl Acad Sci USA 1989;86:232−6.
[20] El Sheikha AF, Condur A, Métayer I, Le Nguyen DD, Loiseau G and Montet D. Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: an application to *Physalis* fruits from Egypt. Yeast 2009;26:567−73.
[21] Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389−402.
[22] Tamura K, Stecher G, Peterson D, Filipski A and Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30:2725−9.
[23] Jeyaram K, Tamang JP, Capece A and Patrizia Romano P. Geographical markers for *Saccharomyces cerevisiae* strains with similar technological origins domesticated for rice-based ethnic fermented beverages production in North East India. Antonie van Leeuwenhoek 2011;100:569−78.
[24] Lee AC and Fujio Y. Microflora of banh men, a fermentation starter from Vietnam. World J Microbiol Biotechnol 1999;15:57−62.
[25] Xie GF, Li WJ, Lu J, Cao Y, Fang H and Zou HJ. Isolation and identification of representative fungi from Shaoxing rice wine wheat qu using apolyphasic approach of culture-based and molecular-based methods. J Institute Brew 2007;113:272−9.
[26] Jeyaram K, Mohendro Singh W, Capece A and Romano P. Molecular identification of yeast species associated with 'Hamei' − a traditional starter used for rice wine production in Manipur. India. Int J Food Microbiol 2008;124: 115−25.
[27] Lv XC, Weng X, Huang RL, Wen Z, Rao PF and Ni L. Research on biodiversity of yeasts associated with Hongqu glutinous rice wine starters and the traditional brewing process. J Chinese Institute Food Sci Technol 2012;12:182−90.

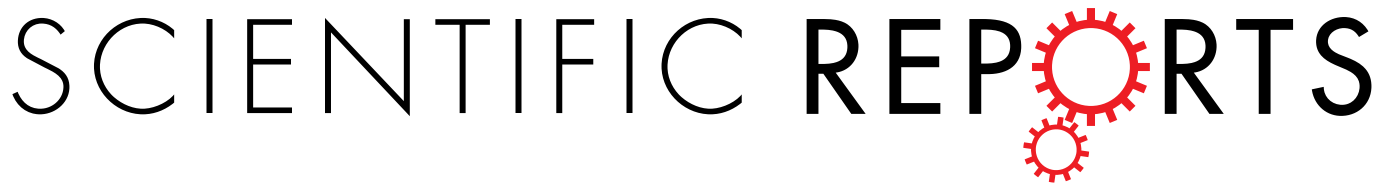
[28] Xu CL, Huang XL, Wen Z, Rao PF and Ni L. Yeast diversity of traditional alcohol fermentation starters for Hong-Qu glutinous rice wine brewing, revealed by culture-dependent and culture-independent methods. Food Control 2013;34:183−90.

[29] Thanh VN, Mai LT and Tuan DA. Microbial diversity of traditional Vietnamese alcohol fermentation starters (banh men) as determined by PCR mediated, DGGE. Int J Food Microbiol 2008;128:268−73.

[30] Ishii K and Fukui M. Optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR. Appl Environ Microbiol 2001;67:3753−5.

[31] Katano T and Fukui M. Molecular inference of dominant picocyanobacterial populations by denaturing gradient gel electrophoresis of PCR amplified 16S rRNA gene fragments. Phycol Res 2003;51:71−6.

[32] Kafili T, Razavi SH, Djomeh ZE, Naghavi MR, Álvarez-Martín P and Mayo B. Microbial characterization of Iranian traditional Lighvan cheese over manufacturing and ripening via culturing and PCR-DGGE analysis: identification and typing of dominant lactobacilli. European Food Res Technol 2009;229:83−92.



OPEN

Analysis of bacterial and fungal communities in *Marcha* and *Thiat*, traditionally prepared amylolytic starters of India

Received: 24 May 2017
Accepted: 29 August 2017
Published online: 08 September 2017

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Marcha **and** ***thiat*** **are traditionally prepared amylolytic starters use for production of various ethnic alcoholic beverages in Sikkim and Meghalaya states in India. In the present study we have tried to investigate the bacterial and fungal community composition of** ***marcha*** **and** ***thiat*** **by using high throughput sequencing. Characterization of bacterial community depicts phylum** ***Proteobacteria*** **is the most dominant in both** ***marcha*** **(91.4%) and** ***thiat*** **(53.8%), followed by** ***Firmicutes*****,** ***and Actinobacteria*****. Estimates of fungal community composition showed** ***Ascomycota*** **as the dominant phylum. Presence of** ***Zygomycota*** **in** ***marcha*** **distinguishes it from the** ***thiat*****. The results of NGS analysis revealed dominance of yeasts in** ***marcha*** **whereas molds out numbers in case of** ***thiat*****. This is the first report on microbial communities of traditionally prepared amylolytic starters of India using high throughput sequencing.**

Traditional practice of sub-culturing by back-sloping and preservation of essential native microbiota consisting of consortia of yeasts, molds and bacteria, in the form of dry, flattened, or round balls, for alcoholic beverages production in South-East Asia including the Himalayan regions of India, Nepal, Bhutan, and China is the worth wisdom of the ethnic people for centuries[1]. Some common and uncommon amylolytic starters in Asia are *marcha* of India, Nepal, and Bhutan, *hamei*, *humao*, *thiat*, *phab* of India, *men* of Vietnam, *bubod* of the Philippines, *chiu/chu* of China and Taiwan, *loogpang* of Thailand, *ragi* of Indonesia, *nuruk* of Korea, *mae/dombae/buh/puh*in Cambodia, etc.[2–7] Traditionally prepared Asian amylolytic starters have consortia of mixed micrbiota representing filamentous molds, yeast and bacteria[1–3], hence many researchers have studied the fungal, yeast and bacterial populations in Asian starter cultures, commonly based on culture-dependent techniques including phenotypic and 16S rRNA sequencing, and isolated and identified filamentous molds *Absidia corymbifera*, *Amylomyces rouxii*, *Botryobasidium subcoronatum*, *Mucor circinelloides* forma *circinelloides*, *Mucor hiemalis*, *Rhizopus oryzae*, *Rhi. microsporus*, *Rhi. chinensis*, and *Rhi. stolonifer*, *Xeromyces* bisporus[5,8,9]; yeasts *Candida glabrata*, *C. tropicalis*, *Clavispora lusitaniae*, *Issatchenkia* sp., *Pichia anomala*, *P. ranongensis*, *P. burtonii*, *Saccharomycopsis fibuligera*, *Sm. capsularis*, *Saccharomyces cerevisiae*, *Sacch. Bayanus*[5,9–13]; and bacteria *Acetobacter orientalis*, *A. pasteurianus*, *Bacillus amyloliquefaciens*, *B. circulans*, *B. sporothermodurans*, *B. subtilis*, *Pediococcus pentosaceus*, *Lactobacillus bifermentans*, *Lb. brevis*, *Lb. plantarum*, *Weissella confusa*, *W. paramesenteroides*[5,14–16].

Introduction of culture-independent methods and it's applicability in food microbiology[7,17], has been a motivation for few researchers to profile the microbial community structure of some Asian starter cultures using PCR-DGGE, pyrosequencing, etc. which is suggestive to provide more insight into the microbial diversity of ethnic starters[3,5,18–22]. Rapid evolution in next generation sequencing (NGS) technologies has enabled researchers to have increased accuracy, throughput, with reasonably low cost and in relatively short period of time[17,23]. However, there are still a limited number of studies, characterizing the microbial community composition of fermented foods such as cheese[24–26], kefir grains[27], some ethnic Asian fermented foods[28–31]. Furthermore, the information on the community composition of Asian starter culture is rudimentary and needs in depth exploration using cutting edge technologies[7].

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Figure 1. Traditionally prepared amylolytic starter cultures (**a**) *Marcha* and (**b**) *Thiat*.

	Chao1	Goods coverage	Shannon	Simpson
a) Bacterial				
Marcha	1520.925	0.998902539	4.01115959	0.866763863
Thiat	4256.838	0.997475969	5.489325073	0.940199394
b) Fungal				
Marcha	5.25	0.75	2.25	0.78125
Thiat	5	1	1.802366931	0.671398892

Table 1. Alpha diversity estimation. Non parametric alpha diversity was calculated for ethnic amylolytic starter cultures *marcha* and *thiat*.

In present study, we attempted to profile the microbial community composition of *marcha* and *thiat*, traditionally prepared ethnic starter cultures of India using targeted amplicon sequencing. We selected two different traditionally prepared amylolytic starter cultures from two regions in India, *marcha* (Fig. 1a) from Sikkim (www.sikkim.gov.in) and *thiat* (Fig. 1b) from Meghalaya (www.megtourism.gov.in). *Marcha* is prepared from soaked rice with some wild herbs ((*Plumbago zeylanica*, *Buddleja asiatica* and *Vernonia cinerea*), ginger and red dry chili, 1–2 % of previously prepared *marcha* powder as an inoculum, crushed in a wooden mortal by wooden pestle, mised and dough are made into round to flatted cakes of different size and shape. Cakes are covered with fern fronds (*Glaphylopteriolopsis erubeseens*), fermented at room temperature for 24 h, sun dried for 3–5 days and are used as amylolytic starters for production of cereal-based ethnic fermented beverages such as *kodo ko jaanr*, *bhaati jaanr*, *raksi*, etc.[2] During *thiat* preparation, soaked glutinous rice is grinded with leaves and roots of wild plant *Amomum aromaticum*, 1–2% of old *thiat*, mixed and made into a dough by adding water. Flat to round balls are made and fermented for 1–3 days. The freshly prepared *thiat* balls are sun dried for 3–5 days. It is used to ferment alcoholic beverage locally called *kiad* in Meghalaya[2]. Fermentation process involved in preparation of these starters is unconditional and may harbor both bacterial and fungal communities as consortia. Therefore, we aimed to explore the bacterial and fungal (filamentous molds and yeasts) communities in *marcha* and *thiat*. This is the first report on complete microbial community profile of traditionally prepared amylolytic starters of India using NGS technique.

Results

Characterizing microbial diversity. High throughput sequencing and quality trimming of 16S rRNA and ITS gene yielded ~0.85and ~0.29 million quality reads in both *marcha* and *thiat*, respectively, which was used for subsequent analysis. Taxonomic assignment of sequences with the reference database resulted into 5,015 operational taxonomic units (OTUs). The average Good's coverage of both the samples of *marcha* and *thiat* for 16Sr RNA amplicon sequencing was found to be 99.08% ± 0.1% (mean ± SD) whereas for ITS region was recorded as 87.5% ± 17.6% (mean ± SD) indicating majority of the diversity was captured.

The estimates of alpha diversity indices revealed significant differences between *marcha* and *thiat* when computed for both the bacterial and fungal diversity (Table 1a and b). The bacterial species richness was found to be higher in *thiat* (4256.83) than *marcha* (1520.92), in contrast, fungal species richness depicts higher in *marcha* (5.25) over *thiat* (5.0). Significant variations were also noticed in non-parametric shannon index for bacterial communities in *thiat* (5.48) and *marcha* (4.01). Shannon index for fungal communities follow the reverse trend with *marcha* (2.25) and *thiat* (1.80). This observation is suggestive of higher bacterial diversity in *thiat* while *marcha* showed higher fungal diversity.

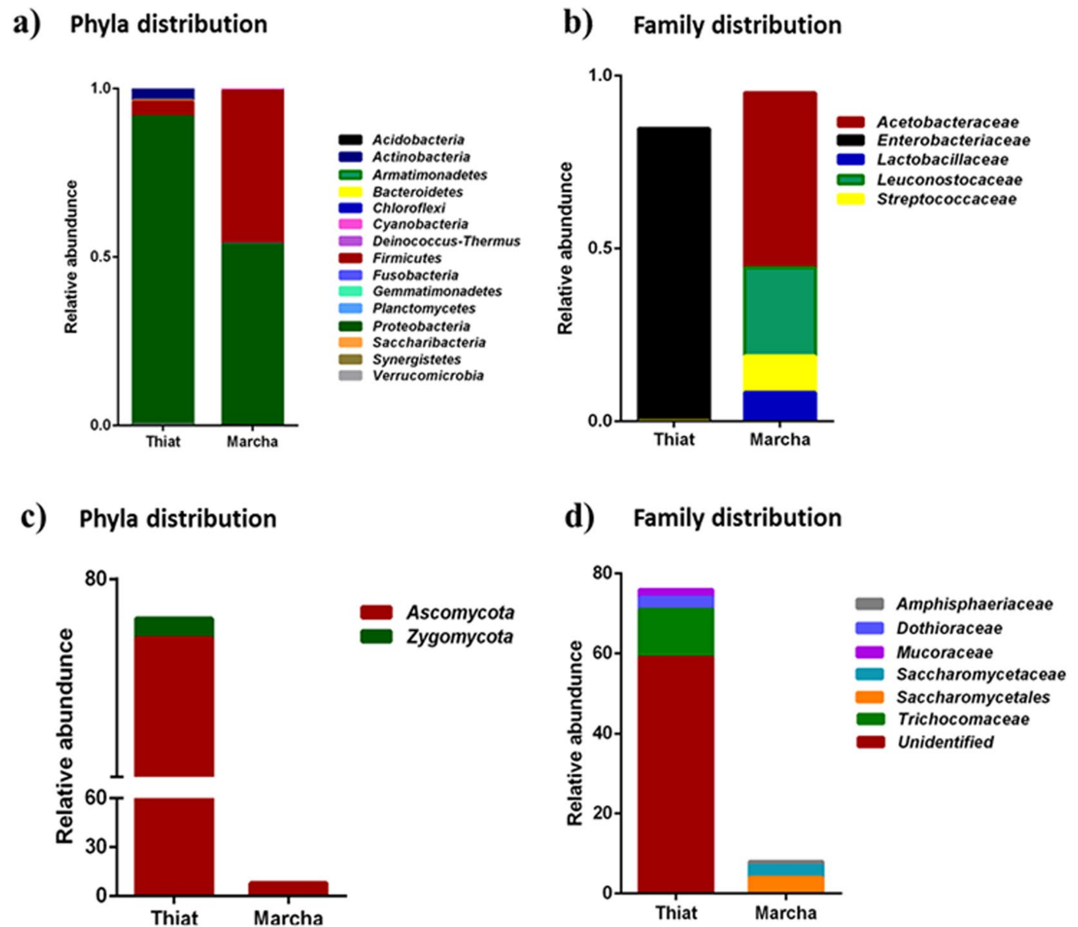

Figure 2. Taxa distributions of phylum and family at different phylogenetic level in *thiat* and *marcha*. (**a**) bacterial phyla; (**b**) bacterial family; (**c**), fungal phyla and (**d**) fungal family.

Bacterial community profile of *thiat* and *marcha*. 16S rRNA gene amplicon sequencing yielded 15 bacterial phyla in *thiat* and *marcha*, respectively (Fig. 2a). In *thiat* bacterial phyla distributions were *Proteobacteria* (91.4%), *Actinobacteria* (4%), *Firmicutes* (4%) and the rest (0.6%) constituted the minor phyla *Cyanobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Fusobacteria*, *Planctomycetes*, *Deinococcus-Thermus*, *Chloroflexi*, *Synergistetes*, *Acidobacteria*, *Saccharibacteria*, *Gemmatimonadetes*, *Armatimonadetes*. In *marcha* the phyla distributions of bacteria were *Proteobacteria* (53.8%), *Firmicutes* (45.4%) and other minor phyla were 0.8% constituting *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Fusobacteria*, *Planctomycetes*, *Deinococcus-Thermus*, *Chloroflexi*, *Synergistetes*, *Acidobacteria*, *Saccharibacteria*, *Gemmatimonadetes*, and *Armatimonadetes*. The abundance of thirteen minor phyla was very less hence percentage of composition was not shown in Fig. 2a. Bacterial phylum *Proteobacteria* was found to outnumber other bacterial phyla in *thiat* whereas *marcha* was found to constitute *Proteobacteria* and *Firmicutes* as major phyla.

At family level, OTUs with ≥1% abundance were filtered which differed quantitatively between *thia*t and *marcha* (Fig. 2b). The family level distributions of bacteria in *thiat* were *Enterobacteriaceae* (84.6%), *Microbacteriaceae* (3.24%), *Enterococcaceae* (2.47%), *Clostridiaceae* (1.13%) *Neisseriaceae* (0.87%) and *Oxalobacteraceae* (0.59%) (Fig. 2b). Whereas the family level of bacterial distributions in *marcha* were *Acetobacteraceae* (50.6%), *Leuconostocaceae* (25.5%), *Streptococcaceae* (10.5%), *Lactobacillaceae* (8.38%), *Burkholderiaceae* (2.13%) and *Staphylococcaceae* (0.54%) (Fig. 2b).

At the genus level, OTUs with ≥1% abundance were filtered (Fig. 3a,b), which retained 18 differentially abundant genera in both samples of *marcha* and *thiat*. Distribution of bacterial genera in *marcha* were *Acetobacter* (52.6%), *Fructobacillus* (21.1%), *Lactococcus* (10.3%), *Lactobacillus* (8.4%), *Leuconostoc* (4.0%) (Fig. 3a), *Burkholderia* (2.1%) and *Gluconacetobacter* (1.4%). Genera in *thiat* were *Pantoea* (32.4%), *Cronobacter* (21.4%), *Escherichia-Shigella* (15.5%), *Enterobacter* (13.1%), *Citrobacter* (4.2%) (Fig. 3b), *Salmonella* (3.2%), *Serratia* (2.8%), *Enterococcus* (2.5%), *Curtobacterium* (2.2%), *Kluyvera* (1.6%) and *Clostridium* (1.1%). The composition percentage of bacterial genera which was less than 3.9% was not shown in Fig. 3a,b.

Fungal (filamentous molds and yeasts) composition in *thiat* and *marcha*. Fungal ITS gene sequencing and taxonomic analysis demonstrated the predominance of yeast phylum *Ascomycota* (98.6%) in *thiat*, whereas the distribution of filamentous phyla *Zygomycota* was only 1.4% (Fig. 2c). However, in *marcha* only yeast phylum *Ascomycota* constituted the fungal diversity (Fig. 2c). Filamentous mold phylum was not detected

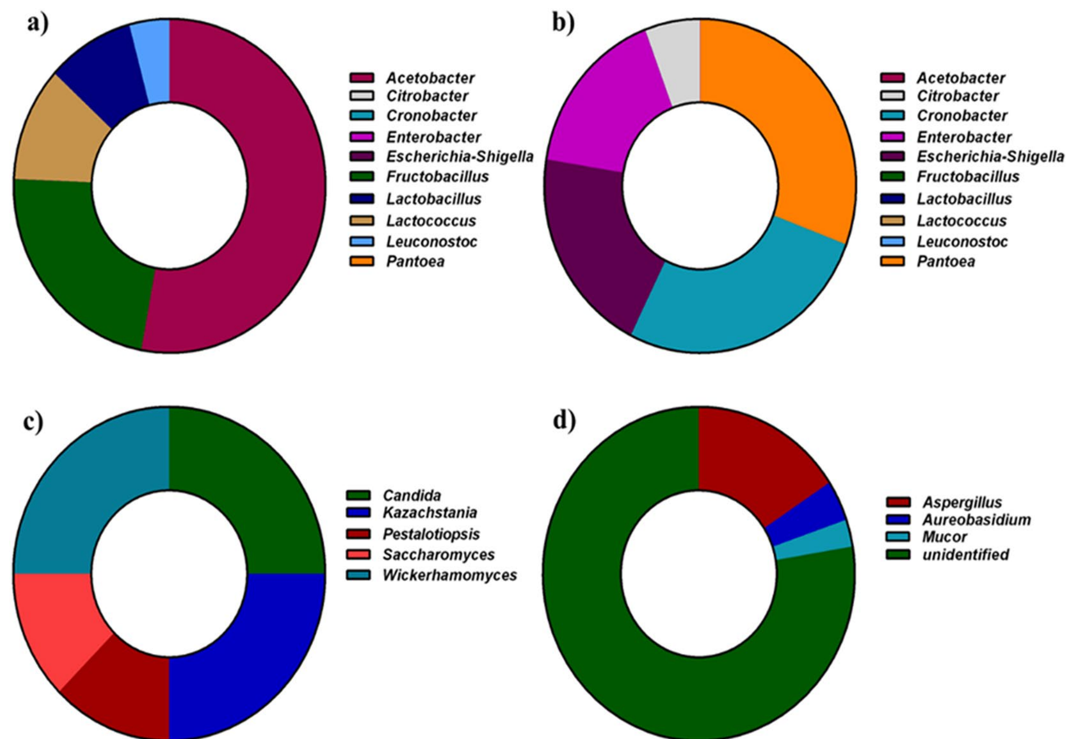

Figure 3. Taxa distributions of genus at different phylogenetic level. (**a**) bacterial genera in *marcha*; (**b**) bacterial genera in *thiat*; (**c**) fungal genera in *marcha* and (**d**) fungal genera in *thiat*.

in *marcha*. Distributions of fungi (filamentous molds and yeasts) at the family level in *thiat* were *Trichocomaceae* (15.7%), *Dothioraceae* (3.94%), *Mucoraceae* (2.63%) and unidentified fungi (77.73%). Whereas the distributions of yeasts at the order/family level in *marcha* were *Saccharomycetales* (50%), *Saccharomycetaceae* (37.5%) and *Amphisphaeriaceae* (12.5%). (Fig. 2d). Distributions of yeasts genera in *marcha* were *Wickerhamomyces* (25%), *Candida* (25%), *Kazachstania* (25%), *Saccharomyces* (12.5%) and *Pestalotiopsis* (12.5%) (Fig. 3c). The filamentous mold genera distribution in *thiat* were *Aspergillus* (15.7%), *Aureobasidium* (3.9%) and *Mucor* (2.7%) and unidentified genera (77.7%) (Fig. 3d). The unidentified genera represented the yeast phylum *Ascomycota* in *thiat*. The sequence reads showed the species of filamentous molds were *Aspergillus penicillioides*, *Aureobasidium pullulans* and *Mucor circinelloides*, whereas the yeasts species were *Wickerhamomyces anomalus*, *Candida quercitrus* and *Kazachstania exigua* (data not shown).

Discussion

Our study provides comprehensive microbial diversity analysis using deep sequencing approach of ethnic amylolytic starter from India. Quantitative differences were noted for the presence of bacterial and fungal taxa among *marcha* and *thiat*; which could be the consequence of differences in the preparation, incubation period and most importantly the type of preservations. Alpha diversity estimation of amylolytic starters *marcha* and *thiat* using species richness and non-parametric Shannon index suggested higher bacterial diversity in *thiat* while *marcha* shows the higher assemblage of fungal diversity with dominance of yeast phylum *Ascomycota*. Persistence of higher fungal diversity in *marcha* is determinant factor suggesting the higher acidic conditions of *marcha*; in contrast, higher bacterial diversity of *thiat* depicts the faster turnover from acidic to alkali with the presence of acid neutralizing bacterial taxa[32].

Acetobacter, *Fructobacillus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Burkholderia*, and *Gluconacetobacter* were the predominant bacterial genera in *marcha*. Higher proportion of *Acetobacter* was possibly due to its retention and enrichment during fermentation. We observed relatively lower proportion of *Streptococcus* and *Lactococcus* than *Lactobacilli;* as *Lactobacilli* have high acid tolerance over former two[33]. Though some species of *Lactococcus* have low acid tolerance, however, they could be isolated from raw milk and were found flourishing during the early stage of fermentation[24]. This supports the lower abundance of *Lactococcus* than *Lactobacillus* as seen in our samples. Another interesting observation was absence of *Pediococcus* in bacterial community profile which was otherwise present as a one of the dominant genus in earlier report by culture dependent methods in *marcha*[10, 16]. Furthermore, since there is no earlier report on microbial composition based on culture dependent or culture-independent methods of *thiat* the present study describe microbial diversity of *thiat* using NGS method as its first report. *Pantoea*, *Cronobacter*, *Escherichia*, *Shigella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Serratia*, and *Enterococcus* depicts most dominant bacterial genera of *thiat* each comprised over 0.1% of total bacterial sequences. Significantly varied microbial composition among *thiat* and *marcha* is a clear indication of differences in amylolytic starters. Genus *Enterobacter* was also detected in Mexican alcoholic beverages speculated

to originate from the bacterial contamination in raw milk and they subsequently decreased during the fermentation process[34]. The lactic acid bacterium such as *Lb. plantarum* seemed to be one factor for the good quality of the alcoholic beverages, as it can perform malolactic fermentation to decrease wine acidity[32] and also produces bacteriocins[35].

Exploration of fungal diversity of ethnic amylolytic starters suggested higher abundance of yeast in *marcha* and *thiat* constitutes for 32.33-fold yeast to the filamentous molds. This observation was in coherence with the earlier report of culture-dependent studies showing the dominance of *Mucor* and *Rhizopus* genera of *Mucorales* in *marcha*[8]. Interestingly no filamentous molds were detected in *marcha* using the applied high throughout sequencing method; the exact reasons for the observed variation in the microbiota have not been identified. This may be due to lower abundance of molds, limited sample size and/or age of the sample and finally also due to inadequate cell lysis which may prevent the release of nucleases[36]. Our study was in accordance to the previous reports describing the exposure of cheese to different external environments such as manufacturing process; geographical region, etc have varied impact on the microbial composition of the end products[28]. Thus, we speculate that the factor of geographic environment including altitudes and climate play a more significant role over the manufacturing process in resulting in the different microbial compositions of the starter culture under study. Some other crucial factors that may affect the composition of microbial communities in fermented amylolytic starters are level of hygiene, quality of the glutinous rice, water, as well as the back slopping technique. In this study three dominant yeasts in *marcha* were *Wickerhamomyces anomalus*, *Candida quercitrus* and *Kazachstania exigua*, followed by *Saccharomyces* and *Pestalotiopsis* which also accompany the findings of ref. 21 by PCR-DGGE method. ITS gene sequences analysis of the *thiat* revealed the existence of *Aspergillus penicillioides*, *Aureobasidium pullulans* and *Mucor circinelloides* as the most dominant filamentous molds in *thiat*. At family level *thiat* shows *Trichocomaceae*, *Dothioraceae*and *Mucoraceae*as the major constituents of fungal community composition emphasizing the significant differences between *thiat* and *marcha* viz differences in starter substrates, preparations, inoculums, consortia, geography, hygiene, preservation technique, caloric values etc.

In the present study *Ascomycota* was dominant in starter cultures of India like in Korean and Chinese starters cultures, which was also reported earlier, based on NGS tools, in Korean alcoholic beverages[3] and in Chinese liquors[37]. We could also expect similar observation in case of *marcha* as it has higher abundance of lactic acid bacteria. *Aspergullus oryzae* has strong secretion of amylases including alpha-amylase, which may accelerate the degradation of grains and provide more nutrients for microbes in alcoholic fermentation[38].

Amylolytic starter culture-making technology reflects the traditional knowledge of the ethnic Indian people on sub-culturing desirable inocula from previous batch to new culture using rice as base substrates by back-sloping method. This technique preserves the consortia of microbial community ranging from filamentous molds, yeasts and bacteria which were co-existed in traditionally prepared amylolytic and alcohol producing starters[7], and also preserves vast biological genetic resources, otherwise, which may be forced to disappear. Fermented beverages produced by using amylolytic starters in India are generally mild-alcoholic (4–5%), sweet taste with several health benefits to the local consumers as high source of calories, some vitamins and minerals[2]. Ethnic fermented beverages and alcoholic drinks have the potential to grow beverage industry if proper scientific and technical support are applied to the existing indigenous practices of home based alcoholic fermentation.

Materials and Methods

Sample collection. Samples of sun-dried amylolytic starters *marcha* and *thiat* were collected immediately after the preparation from local people of Gangtok and Shillong in Sikkim and Meghalaya states of India, respectively. Dry samples were transferred to sterile containers, sealed, and stored at desiccator at room temperature for the further analysis.

Community DNA Extraction. The total community DNA was extracted using ProMega DNA kit (ProMega). 1g of amylolytic starter culture sample was suspended in lysis solution and incubated at 65 °C for 15 min. Subsequently, the RNA was eliminated from the cellular lysate by administering the RNase solution following incubation at 35 °C for 15 min. The residual proteins were removed by adding protein precipitation solution and centrifuged at max speed. Finally, the DNA was precipitated by adding isopropanol, which was purified with two washes of 70% ethanol. The quality of DNA was checked on 0.8% agarose gel and concentration was measured using Nano-DropND-1000 spectrophotometer (Nano Drop technologies, Willington, USA) as described by ref. 39. The DNA was stored at −20 °C until further processing.

Amplicon sequencing. Amongst the nine hypervariable regions of bacterial 16S rRNA gene, we have targeted V4 hyper-variable region[40] to investigate bacterial diversity of *marcha* and *thiat*. The universal 16S rRNA gene primer sets F515 and 806R[41] was used for the amplification of V4 hyper-variable region. Similarly, fungal Internal Transcribed Spacer (ITS) II region was targeted for taxonomic profiling amylolytic starters, which was subjected to amplification using ITS1 and ITS2 primers. The library preparation of both the 16S rRNA and ITS gene amplicons were in accordance with the protocols of Illumina (USA). These amplicon libraries were further processed for sequencing using AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA). The resultant product was screened with the LabChip GX (Perkin Elmer, Waltham, MA, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA). Subsequently, the 16S rRNA and ITS gene library were sequenced on the Illumina MiSeq platform using 2x 250bp chemistry. The sequences obtained from high throughput sequencing effort were submitted to National Center for Biotechnology Information (NCBI) which are available under BioProject ID PRJNA376467.

Bioinformatics analysis. The raw sequences generated from MiSeq platform was assembled using FLASH tool (Fast Length Adjustment of Short reads) a Paired end assembler for DNA sequences[42]. The assembled reads were subjected to quality filtering using via Quantitative Insights into Microbial Ecology (QIIME) 1.8[42]. Sequence

reads were assigned to bacterial and fungal operational taxonomic units (OTUs) by a closed reference-based OTU picking approach by using SILVA and UNITE reference databases, respectively. The OTU picking was carried out using UCLUST method with similarity threshold of 97%[43]. Taxonomic assignments were performed using RDP naïve bayesian classifier[44]. Alpha diversity indices like Chao, Shannon and Simpson were calculated via QIIME after rarefying all samples to the same sequencing depth[45,46].

Data availability. The sequences obtained from high throughput sequencing effort, was submitted to NCBI which are available under Bio Project ID PRJNA376467.

References

1. Tamang, J. P. & Kailasapathy, K. *Fermented foods and beverages of the world*. CRC Press (Eds.), Taylor and Francis Group, New York (2010).
2. Tamang, J. P. *Himalayan fermented foods: microbiology, nutrition and ethnic value*, CRC Press, Taylor and Francis Group, New York (2010).
3. Jung, M. J., Nam, Y. D., Roh, S. W. & Bae, J. W. Unexpected convergence of fungal and bacterial communities during fermentation of traditional Korean alcoholic beverages inoculated with various natural starters. *Food Microbiol.* **30**, 112–23 (2012).
4. Sujaya, I. *et al*. Identification and characterization of yeasts in brem, a traditional Balinese rice wine. *World J. Microbiol. Biotechnol.* **20**, 143–150 (2004).
5. Thanh, V. N., Mai, L. T. & Tuan, D. A. Microbial diversity of traditional Vietnamese alcohol fermentation starters (*banh men*) as determined by PCR-mediated DGGE. *Int. J. Food Microbiol.* **128**, 268–273 (2008).
6. Yamamoto, S. & Matsumoto, T. Rice fermentation starters in Cambodia: cultural importance and traditional methods of production. *Southeast Asian Studies.* **49**, 192–213 (2011).
7. Tamang, J. P., Watanabe, K. & Holzapfel, W. H. Review: Diversity of microorganisms in global fermented foods and beverages. *Front. Microbiol.* **7**, 377, doi:10.3389/fmicb.2016.00377 (2016).
8. Tamang, J. P., Sarkar, P. K. & Hesseltine, C. W. Traditional fermented foods and beverages of Darjeeling and Sikkim - a review. *J. Sci. Food Agric.* **44**, 375–385 (1988).
9. Dung, N. T. P., Rombouts, F. M. & Nout, M. J. R. Functionality of selected strains of moulds and yeasts from Vietnamese rice wine starters. *Food Microbiol.* **23**, 331–340 (2006).
10. Tamang, J. P. & Sarkar, P. K. Microflora of murcha: an amylolytic fermentation starter. *Microbios.* **81**, 115–122 (1995).
11. Tsuyoshi, N. *et al*. Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation. *Int. J. Food Microbiol.* **99**, 135–146 (2005).
12. Jeyaram, K., Mohendro Singh, W., Capece, A. & Romano, P. Molecular identification of yeast species associated with 'Hamei"- a traditional starter used for rice wine production in Manipur, India. *Int. J. Food Microbiol.* **124**, 115–125 (2008).
13. Jeyaram, K., Tamang, J. P., Capece, A. & Romano, P. P. Geographical markers for *Saccharomyces cerevisiae* strains with similar technological origins domesticated for rice-based ethnic fermented beverages production in North East India. *Antonie van Leeuwenhoek.* **100**, 569–578 (2011).
14. Dung, N. T. P., Rombouts, F. M. & Nout, M. J. R. Characteristics of some traditional Vietnamese starch-based rice wine starters (*Men*). *LWT-Food Sci. Technol.* **40**, 130–135 (2007).
15. Hesseltine, C. W. & Ray, M. L. Lactic acid bacteria in murcha and ragi. *J. Appl. Bacteriol.* **64**, 395–401 (1988).
16. Tamang, J. P. *et al*. Lactic acid bacteria in *Hamei* and *Marcha* of North East India. *Indian J. Microbiol.* **47**, 119–125 (2007).
17. Cocolin, L., Alessandria, V., Dolci, P., Gorra, R. & Rantsiou, R. Culture independent methods to assess the diversity and dynamics of microbiota during food fermentation. *Int. J. Food Microbiol.* **167**, 29–43 (2013).
18. Chao, S. H. *et al*. Microbial diversity analysis of fermented mung beans (Lu-Doh-Huang) by using pyrosequencing and culture methods. *PLOS ONE.* **8**(5), e63816 (2013).
19. Chen, B., Wu, Q. & Xu, Y. Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese *Maotai*-flavor liquor. *Int. J. Food Microbiol.* **179**, 80–84 (2014).
20. Lv, X. C., Huang, X. L., Zhang, W., Rao, P. F. & Ni, L. Yeast diversity of traditional alcohol fermentation starters for Hong Qu glutinous rice wine brewing, revealed by culture-dependent and culture-independent methods. *Food Control.* **34**, 183–190 (2013).
21. Sha, S. P., Anupma, A., Pradhan, P., Prasad, G. S. & Tamang, J. P. Identification of yeasts by PCR-mediated DGGE in *marcha*, an ethnic amylolytic starter of India. *J. Ethnic Foods.* **3**, 292–296 (2016).
22. Zhu, L. *et al*. BOX-PCR and PCR-DGGE analysis for bacterial diversity of a naturally fermented functional food (Enzyme). *Food Bioscience.* **5**, 115–122 (2014).
23. Mayo, B. *et al*. Impact of next generation sequencing techniques in food microbiology. *Curr. Genomics.* **15**(4), 293–309 (2014).
24. Alegría, Á., Szczesny, P., Mayo, B., Bardowski, J. & Kowalczyk, M. Biodiversity in Oscypek, a traditional Polish cheese, determined by culture-dependent and-independent approaches. *Appl. Environ. Microbiol.* **78**, 1890–8 (2012).
25. Quigley, L. *et al*. High-throughput sequencing for detection of subpopulations of bacteria not previously associated with artisanal cheeses. *Appl. Environ. Microbiol.* **78**, 5717–23 (2012).
26. Ercolini, D., De Filippis, F., La Storia, A. & Iacono, M. A "remake" of the microbiota involved in the production of water buffalo mozzarella cheese by high throughput sequencing. *Appl. Environ. Microbiol.* **78**, 8142–8145 (2012).
27. Bourrie, B. C. T., Willing, B. P. & Cotter, P. D. The microbiota and health promoting characteristics of the fermented beverage kefir. *Front. Microbiol.* **7**, 647 (2016).
28. Nam, Y. D., Lee, S. Y. & Lim, S. I. Microbial community analysis of Korean soy- bean pastes by next-generation sequencing. *Int. J. Food Microbiol.* **155**, 36–42 (2012).
29. Romi, W., Ahmed, G. & Jeyaram, K. Three-phase succession of autochthonous lactic acid bacteria to reach a stable ecosystem within 7 days of natural bamboo shoot fermentation as revealed by different molecular approaches. *Mol. Ecol.* **24**, 3372–3389 (2015).
30. Keisam, S., Romi, W., Ahmed, G. & Jeyaram, K. Quantifying the biases in metagenome mining for realistic assessment of microbial ecology of naturally fermented foods. *Sci. Rep.* **6**, 34155 (2016).
31. Zhang, J. *et al*. Metagenomic approach reveals microbial diversity and predictive microbial metabolic pathways in Yucha, a traditional Li fermented food. *Sci. Rep.* **6**, 32524 (2016).
32. Kosseva, M., Beschkov, V., Kennedy, J. F. & Lloyd, L. L. Malolactic fermentation in Chardonnay wine by immobilized. *Lactobacillus casei* cells. *Process Biochem.* **33**, 793–797 (1998).
33. Rogosa, M., Mitchell, J. A. & Wiseman, R. F. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. *J. Bacteriol.* **62**, 132 (1951).
34. Escalante, A. *et al*. Analysis of bacterial community during the fermentation of pulque, a traditional Mexican alcoholic beverage, using apolyphasic approach. *Int. J. Food Microbiol.* **124**, 126–34 (2008).
35. Navarro, L., Zarazaga, M., Ruiz-Larrea, F., Torres, C. & Saenz, J. Bacteriocin production by lactic acid bacteria isolated from Riojared wines. *J. Appl. Microbiol.* **88**, 44–51 (2000).

36. Dolci, P., Alessandria, V., Rantsiou, K. & Cocolin, L. Advanced methods for the identification, enumeration, and characterization of microorganisms in fermented foods. In *Advances in Fermented Foods and Beverages: Improving Quality, Technologies and Health Benefits*. Elsevier (2015).
37. Li, X. R. *et al*. Bacterial and fungal diversity in the traditional Chinese liquor fermentation process. *Int. J. Food Microbiol.* **146**, 31–7 (2011).
38. Li, H. Simultaneous saccharification and fermentation of broken rice: an enzymatic extrusion liquefaction pretreatment for Chinese rice wine production. *Bioprocess Biosyst. Eng.* **36**, 1141–1148 (2013).
39. Kumbhare, S. V. *et al*. Insights into diversity and imputed metabolic potential of bacterial communities in the continental shelf of Agatti Island. *PLoS One.* **10**, 0129864 (2015).
40. Bartram, A. K., Lynch, M. D., Stearns, J. C., Moreno-Hagelsieb, G. & Neufeld, J. D. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Appl. Environ. Microbiol.* **77**, 3846–3852 (2011).
41. Caporaso, J. G. *et al*. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods.* **7**, 335–336 (2010).
42. Masella, A. P., Bartram, A. K., Truszkowski, J. M., Brown, D. G. & Neufeld, J. D. PANDA seq: paired-end assembler for illumina sequences. *BMC Bioinformatics* **13**, 31 (2012).
43. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* **26**, 2460–2461 (2010).
44. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**, 5261–5267 (2007).
45. Bokulich, N. A., Lucy Joseph, C. M., Allen, G., Benson, A. K. & Mills, D. A. Next-generation sequencing reveals significant bacterial diversity of Botrytized wine. *PLoS ONE.* **7**, e36357 (2012).
46. Blaalid, R. *et al*. ITS1 versus ITS2 as DNA metabarcodes for fungi. *Mol. Ecol. Resour.* **13**, 218–224 (2013).

Acknowledgements

Authors gratefully acknowledged the financial support of Department of Biotechnology, New Delhi for research project and DAILAB (DBT-AIST International Laboratory for Advanced Biomedicine), Bioinformatics Centre of DBT.

Author Contributions

S.P.S., A.A. and P.P. contributed to this present work as co-authors that are a part of their research work. K.J. and A.S. helped and assisted in all the molecular work and NGS (Bioinformatics and statistical) analyses. A.S., Y.S. and J.P.T. have framed this research paper along with all the authors involved. All authors critically revised, read and approved the final manuscript with approval of J.P.T.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

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Indian Journal of Traditional Knowledge
Vol. 17 (1), January 2018, pp. 184-190

Traditional skill of ethnic people of the Eastern Himalayas and North East India in preserving microbiota as dry amylolytic starters

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Received 09 March 2017, revised 28 July 2017

Preparation of ethnic fermented beverages using dry amylolytic starters is an integral part of socio-cultural practice of different ethnic groups of people residing in the Eastern Himalayan region of Nepal, Bhutan and India including North-East India. Alcoholic beverages are produced by traditional fermentation using specific amylolytic starters, which are prepared in different ways by diverse ethnic groups. This study is aimed at documenting the traditional skill of various ethnic groups of people of North East India and the Eastern Himalayas in preserving microbiota as dry amylolytic starters generally used for preparation of alcoholic beverages.

Keywords: Alcoholic beverage, Amylolytic starter, Ethnic groups, Eastern Himalayas, North East India.

IPC Int. Cl.[8]: C12G, C12C 12/04, C12G 3/08, C12H 3/00, G01N 33/14, C12C 12/00, C12C 5/00

The geographical extent of Eastern Himalayas comprises eastern Nepal, Indian states of Sikkim, Arunachal Pradesh and Darjeeling hills, and a mountainous country Bhutan[1]. North East India is comprised of the cluster of eight states namely Assam, Nagaland, Sikkim and Tripura. More than 137 different ethnic groups reside in these regions[1]. The consumption of alcoholic beverages, prepared by fermentation using amylolytic starter inocula commonly referred to as amylolytic starter, is a common practice among the people of North East India and the Eastern Himalayas. The amylolytic starter is a round to flattened ball of mixed dough containing various wild and domesticated plant parts[2] and amylolytic and alcohol-producing yeasts, starch degrading moulds and lactic acid bacteria[3,4]. Varieties of ethnic amylolytic starters are prepared such as *marcha* of Nepal, Sikkim and the Darjeeling hills in India, *pho* of Bhutan, *emao/humao* of Assam; *xaaz pitha* and *modor pitha* of Assam, *hamei* of Manipur, *thiat* of Meghalaya, *chowan* of Tripura, *kherie/khekhrii* of Nagaland, *Pee*, *paa, phut* and *phab* of Arunachal Pradesh, and *dawdim* of Mizoram (Fig. 1). This paper aims to give extensive information on the indigenous knowledge of preparation process of various amylolytic starters found in different regions of the Eastern Himalayas and North East India.

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Locations of documentation and data collection

Documentation work was carried out for a period of 12 months from December 2015 till November 2016. Data collection was done based on structured questionnaire, interviewing the people involved in traditional preparation of amylolytic starters, personally analyzing the preparation procedures, collection of plant samples and their identification from Botanical Survey of India, Sikkim circle, Gangtok. The study was done in Basilakha village of Sikkim; Dharan, Dhankutta and Hiley districts of Eastern Nepal; Napchey village of Dhonakha, Bhutan; Kokrajhar, Jorhat, Sivsagar, and Moran district of Assam; Kohima of Nagaland and West Siang and Nirjuli district of Arunachal Pradesh. The interviewees were local people of the villages visited and had proper knowledge of preparation of the starter cultures.

Results and discussion

Traditional preparation process of amylolytic starters

Marcha

Marcha is a dry flat creamy white solid ball-like starter of different size and shape traditionally prepared in Sikkim and the Darjeeling hills in India (Fig. 1A), Bhutan (Fig. 1B) and Nepal (Fig. 1C),

Fig. 1 — Amylolytic starter culture of the Eastern Himalayas and North East India- (A): *Marcha* of Sikkim and the Darjeeling hills; B): *Marcha* of Bhutan; (C): *Marcha* of Nepal; (D): *Pho* of Bhutan; (E): *Emao/humao* of Assam; (F): *Xaaz pitha* of Assam; (G): *Modor pitha* of Assam; (H): *Hamei* of Manipur; (I): *Thiat* of Meghalaya; (J): *Chowan* of Tripura; (K): *Kherie/Khekhrii* of Nagaland; (L): *Pee* of Arunachal Pradesh; (M): *Phut* of Arunachal Pradesh; (N): *Paa* of Arunachal Pradesh; (O): *Phab* of Arunachal Pradesh; (P): *Dawdim* of Mizoram.

which is used to ferment starchy material into various ethnic fermented beverages and alcoholic drinks. In the South and eastern parts of Bhutan, *marcha* is predominantly prepared and used by the ethnic *Nepali* of Bhutan. The preparation process of *marcha* in these regions is almost same.

Indigenous knowledge of preparation of marcha

Glutinous rice (*Oryza sativa)* is soaked in water for 8-10 h (overnight) at an ambient temperature. After soaking rice is crushed in a foot driven heavy wooden mortar pestle. Wild herbs such as roots of *guliyo jara* or *chitu* (*Plumbago zeylanica)* leaves of *bheemsen paate* (*Buddleja asiatica*), flower of *sengreknna* (*Vernonia cinerea*), ginger and red dry chili (2-3 pieces) are crushed and added to the powdered rice. Mixtures are then mixed with water to make a thick paste or dough, from which dough balls of different sizes are made. These balls are then dusted with the old *marcha* which are used as an inoculum. The freshly prepared flattened balls are kept on the leaves of fern *Glaphylopteriolopsis erubescens* (commonly known as *pirey uneu*) and covered with the ferns and fermented at room temperature for 24 h. After fermentation, ferns are removed and balls are collected and dried in the sun or kept at room temperature for about 5 days (Fig. 2). The dried *marcha* balls are then sold in the market.

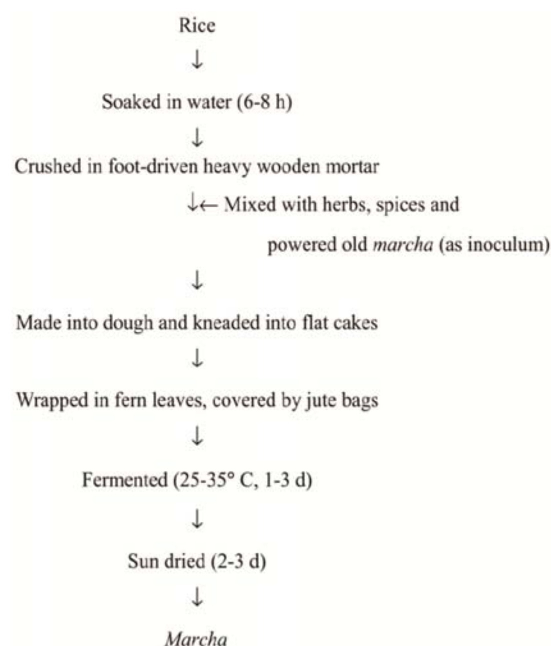

Fig. 2 — Preparation of *marcha* in Sikkim

Mana/Manapu

Mana is a black colored amylolytic starter of Nepal. *Newari* community of Nepal specifically prepares this amylolytic starter to ferment alcoholic distilled liquor, *ayela*, which is very popular alcoholic drinks and is used in special occasions and rituals in

Nepal. Owing to a much easier process of preparation, the use and preparation of *marcha* is replacing the use of *mana* in Nepal, due to which the practice of making *mana* has almost come to an extinction point.

Indigenous knowledge of preparation of mana

During preparation of *mana*, wheat grains are soaked in water over night, boiled for 30 min, drained off excess water, cooled, added dried leaves of *harrah* plant, old *mana* powder and ground in a traditional mortar and pestle. Dough mixture is made into small balls, placed on paddy straw spread on a clean floor, and again covered by paddy straw or straw mat, and fermented for 6-7 days till green moulds appear on the ball. It is dried in the sun to get a black-coloured *mana* and stored for future use.

Pho

Pho or *phab* (Fig. 1D) is a flat dark brown colored cake prepared from powdered maize. This amylolytic starter is prepared indigenously by *Drukpa* community of Bhutan residing in the North Western side of Bhutan. *Pho* is used in preparing the fermented alcoholic drink *Ara*, which is popularly used during the festive seasons in Bhutan.

Indigenous knowledge of preparation of *pho*

The main ingredient used for preparing *pho* is a flower of *bhagham* plant. This flower blooms during October and is white in color and almost 8 inches long (20 cm). The flowers of this plant are sun dried and powdered. During the preparation, powdered maize, rice husks and dried powdered flowers of *bhagham* are taken in a specific ratio of 2:4:3, respectively. Previously prepared *pho* is used as inoculum by back-sloping method and added to the mixtures (Fig. 3). *Pho* stored for more than one year is usually not preferred to be used as a starter culture.

Emao/humao

Emao (Fig. 1E) is amylolytic starter prepared by *Bodo* community of Assam. *Dimasa,* another ethnic community in Assam calls it *humao*. *Emao* is a ball like starter used to ferment glutinous rice to prepare alcoholic beverages of Assam like *zu*/*jou*/*judima*.

Indigenous knowledge of preparation of *emao*

Glutinous rice (*Oryza sativa)* is soaked in water for about 2-3 h and mixed with plants like leaves of banana, *lwkwna*, *dong-phang-rakhep* (*Scoparia dulcis*) and dry barks of *Albizia myriophylla*. These ingredients are then ground together in a wooden mortar pestle (this set of apparatus is locally called *gaihen* and *ual*). The powder is then sieved in a *sandri* (traditional sieve made of bamboo) to which little amount of water is added to make thick paste or dough. Different sizes of small round cakes are made from this dough which are then dusted with the old *emao*, basically used as an inoculum, covered with rice straw and fermented for 2-3 days. After the incubation balls are sundried.

Xaaz pitha

Xaaz pitha (Fig. 1F) is an amylolytic starter produced by *Ahom* community of Assam. *Xaaz pitha* is a ball like starter used to ferment glutinous rice into fermented beverages. Unlike addition of fresh leaves of wild plants during preparation of *emao*, dried leaves are added in preparation of *xaaz pitha*.

Indigenous knowledge of preparation of *xaaz pitha*

Xaaz pitha is traditionally prepared mixed amylolytic dough inocula used as a starter for the preparation of various ethnic alcoholic beverages. Local rice is soaked for 10-12 h and dried leaves and stems of wild herbs are added to the soaked rice. The mixture is then grounded together in an wooden mortar by a pestle. A thick dough is prepared by adding water to the above powdered mixture, from this fresh oval–shaped balls are made and placed on fern leaves (Fig. 4). The freshly prepared *xaaz pitha* is

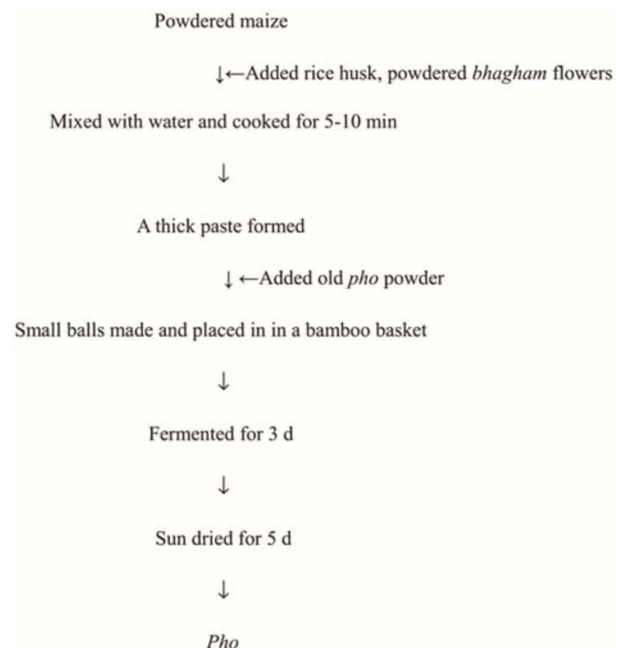

Fig. 3 — Preparation of *pho in* Bhutan

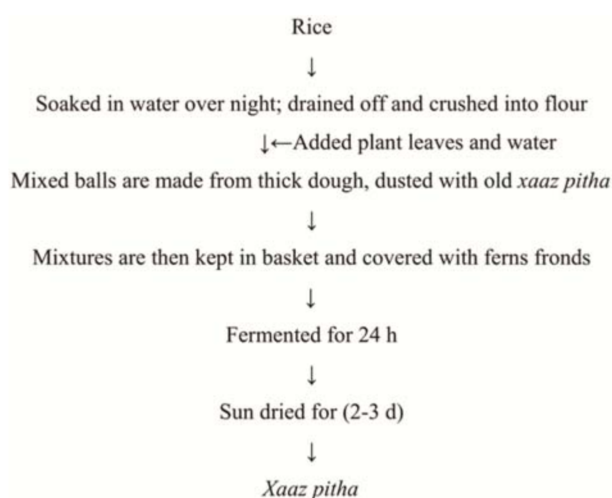

Fig. 4 — Preparation of *xaaz pitha* in Assam

then dried either in the sun or over the fireplace. After a period of about five days they become hard and ready for use.

Modor pitha

Modor pitha (Fig. 1G) is also a starter prepared by *Kachari* community of Assam to ferment rice into alcoholic beverages such as *xaaz pani*.

Indigenous knowledge of preparation of *modor pitha*

Glutinous rice (*Oryza sativa)* is soaked in water for about 2-3 h and mixed with the dried leaves, stem and roots of different types of plants. The above mixture is crushed together in a foot driven wooden mortar pestle (this set of apparatus is locally called *dekhi*). A little amount of water is added to make thick paste, made into small round cakes, dusted with powdered old *modor pitha*, placed on rice straw and then covered with ferns, and fermented for 2-5 days. Once the fermentation process is completed *modor pitha* ball starts to swell. Finally, it becomes hard and is ready to be used as starters for alcoholic beverages.

Hamei

Hamei (Fig. 1H) is a dry, round to flatten, solid ball-like mixed dough inocula used as starter cultures to prepare *atingba*, an alcoholic beverage in Manipur.

Indigenous knowledge of preparation of *hamei*

Hamei is prepared from local varieties of rice which is either soaked in water or used directly without soaking. The rice is mixed with powdered bark of *yangli* (*Albizia myriophylla* Benth.), 1-2 % of old *hamei*, grinded and dough is prepared by adding a small amount of water. The dough is pressed into flat cakes and kept over paddy husk in a bamboo basket, covered by sack clothes and fermented for 2-3 days at room temperature, and then sun dried for 2-3 days (Fig. 5), and is sold in local markets.

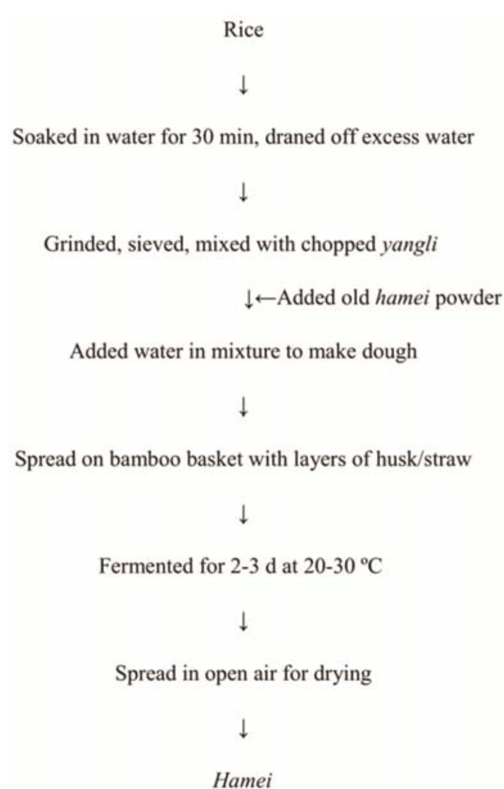

Fig. 5 — Preparation of *hamei in* Manipur

Thiat

Thiat (Fig. 1I) is an amylolytic starter culture used for the preparation of fermented alcoholic beverages in Meghalaya.

Indigenous knowledge of preparation of *thiat*

Glutinous rice is used as a substrate for the preparation of *thiat.* Rice is soaked in water overnight after which it is sun dried for a short period of time to drain off the excess water. Dried leaves and roots of herbs *khaw-iang* (*Amomum aromaticum*) are added to the soaked rice and, and then mixture is made into thick dough by adding water. Flat to round balls are made from the dough and fermented for 1-3 days (Fig. 6). The freshly prepared *thiat* balls are sun dried for 3-5 days.

Chowan

Chowan/chuwan beleb) (Fig. 1J) is a traditionally prepared starter culture of different ethnic tribes in

Fig. 6 — Preparation of *thiat in* Meghalaya

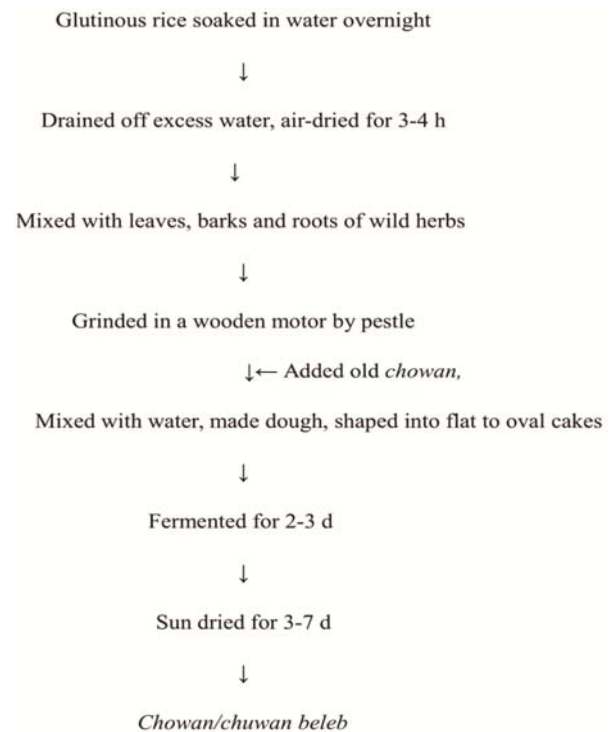

Fig. 7 — Preparation of *chowan* in Tripura

Tripura, and is used for production of local alcoholic beverage *gora bwtwk* and distilled alcoholic drinks *chuwak*.

Indigenous knowledge of preparation of *chowan*

During *chowan* preparation, soaked glutinous rice is mixed with roots, barks and leaves of locally available herbs and powdered old *chowan*. Use of wild herbs during preparation of *chowan* varies from one tribe to other. The mixture is then made into paste by adding water and kneaded into flat and oval cakes of varying sizes and shapes, and fermented for 2-3 days over earthen oven in kitchen (Fig. 7). These freshly prepared *chowan* cakes are then sun dried for 3-7 days and used.

Khrie/Khekhrii

Khrie/khekhrii (Fig. 1K) is an amylolytic starter culture prepared by germinated sprouted rice grains in Nagaland. This is the only amylolytic starter in North-East India which is not prepared by using the old starter through back-sloping method, rather it is prepared by fermenting germinated sprouted-rice grains and then sun-dried to use as dry starters to prepare the local alcoholic drink called *zutho*.

Indigenous knowledge of preparation of *khrie/khekhrii*

Unhulled glutinous rice (*dhan*) is washed with water two to three times and then soaked into water for 2-5 days. It is then kept and covered with *khreihenyii* leaves and allowed to germinate for 2-3 days in summer and 4-5 days in winter. After germination when the rice sprout is about half an inch in length, the sprouted rice is sun dried and powdered (Fig. 8).

Phab

Phab (Fig. 1O) is a flat white colored cake, which is prepared using rice flour. *Tagin* community of Arunachal Pradesh prepares this amylolytic starter traditionally. *Apatani* community and *Nyshing* community of Arunachal Pradesh also prepare similar starter cultures called *pee* (Fig. 1L) and *paa* (Fig. 1N), respectively.

Indigenous knowledge of preparation of *phab*

During *phab* preparation soaked rice is dried and mixed with leaves of some plants *nakail* (*Cinnamomum glanduliferum* Mesissn.), *ctuepatti* (*Cissampelos pareira* L.), *khanoba (Clerodenderon viscosum* Vent.), 5-7 balls of old *phab* are added, made into fine powder, added water and mixed

Fig. 8 — Preparation of *khrie/khekhrii* in Nagaland

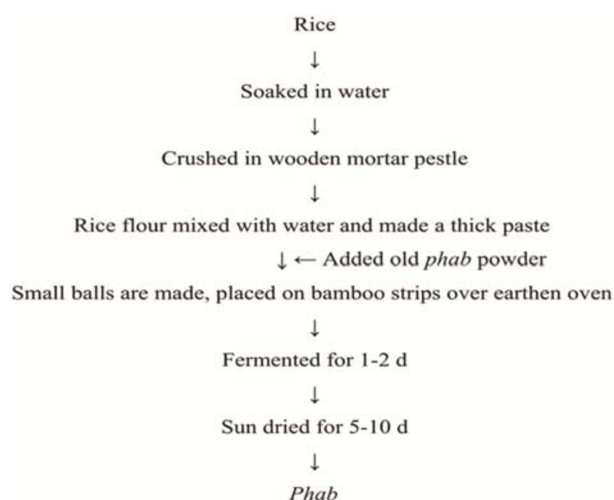

Fig. 9 — Preparation of *phab* in Arunachal Pradesh

thoroughly. The mixture is made into a paste and then small round cakes, wrapped in fresh leaves and placed in bamboo strips over the fireplace and fermented for 1-2 days. Cakes are sun dried for 5-10 days and store up to 6 months (Fig. 9). The preparation process of *phab, pee* and *paa* is almost same except for *paa* where rice is boiled without soaking. No herbs are added during the preparation of *pee*.

Phut

Phut (Fig. 1M) is a flat cake prepared by glutinous rice flour. *Mongpa* community of Arunachal Pradesh prepares *phut* traditionally.

Indigenous knowledge of preparation of *phut*

During *phut* preparation soaked rice is dried and crushed into a fine powder using wooden mortar and pestle. Handful leaves of plant along with cakes of old *phut* are made into fine powder, added to rice flour and mixed thoroughly. The mixture paste is made into small round cakes, wrapped in straw and fermented for 1-2 days. Cakes are sun dried for 3-7 days.

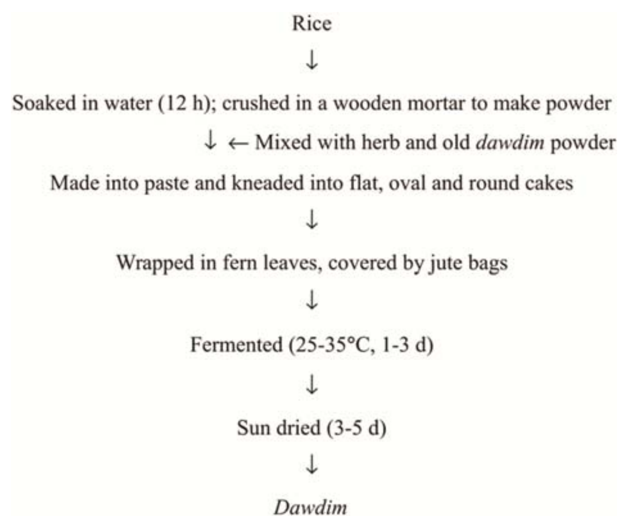

Fig. 10 — Preparation of *dawdim* in Mizoram

Dawdim

Dawdim (Fig. 1P) is a traditionally prepared amylolytic starter of Mizoram used in preparation of local alcoholic beverages.

Indigenous knowledge of preparation of *dawdim*

During preparation, soaked local varieties of rice are crushed to make fine rice flour, mixed with local leaves of herbs with addition of 2 % old *dawdim.* The mixture is then made into paste by adding water and kneaded into flat and oval cakes of varying sizes and shapes, wrapped in fern leaves in bamboo-made baskets, and then covered by jute bags and kept for fermentation above the earthen kitchen oven for 1-3 days. These freshly prepared cakes are sun dried for 3-5 days (Fig. 10).

Socioeconomic importance

The present documentation focuses on the preparation of amylolytic starters, which are used to make fermented beverages. It was observed that the substrate (rice) used for making the amylolytic starters is almost same among the different ethnic groups, except for the *Drukpa* community of Bhutan who used maize instead of rice as the starchy substrate. Amylolytic starters are not just prepared at household level but also at a commercial scale in small villages of North-East states of India and the Eastern Himalayan regions of Nepal and Bhutan. From these small villages the starter cultures are supplied to the local markets and sold at various

prices. Some ethnic groups of people are economically dependent on the preparation of these amylolytic starter cultures. The mountain women have been storing and culturing the functional microorganisms for alcoholic fermentation in the form of dry starter cultures for more than 1000 of years[1]. The producers earn about 60-70 % profit by selling these starters and are one of the major sources of income in the village areas contributing to local economy.

The consortium of microbiota consisting of filamentous moulds, amylolytic and alcohol producing yeasts and species of lactic acid acid is preserved in cereals as starchy bases, as a source of starch, together with the use of glucose-rich wild herbs to supplement the carbon sources for growing microorganisms[1]. Starter culture-making technology reflects the traditional method of sub-culturing desirable inocula from previous batch to new culture using rice as base substrates. This technique preserves the microbial diversity for beverages production. Saccharifying activities are mostly shown by filamentous moulds *Rhizopus* spp. and *Mucor* spp., and yeast *Saccharomycopsis fibuligera* whereas liquefying activities are shown by *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae*[5]. *Rhizopus* spp. and *Sm. fibuligera* degrade cereal starch and produce glucose, and then alcohol-producing yeasts species of *Saccharomyces* and *Pichia* rapidly convert glucose into ethanol[5]. Lactic acid bacteria present in amylolytic starters impart flavor, antagonism and acidification of the substrates[6]. Fermented beverages produced by using amylolytic starters are generally mild-alcoholic (4-5 %), sweet taste with several health benefits to the local consumers as high source of calories, some vitamins and minerals[1,7].

Conclusion

It has been noticed that majority of the young generations do not know ethnic foods, their culinary practices and processing method. Native microorganisms with vast biological genetic resources, which are associated with ethnic fermented foods, are forced to disappear. These fermented food and beverages have the potential to grow in a small medium sized industry if proper scientific and technical support is extended to the existing indigenous practices of home based fermentation.

References

1 Tamang JP, *Himalayan Fermented Foods: Microbiology, Nutrition and Ethnic value*, (CRC Press, Taylor and Francis Group, USA, New York), 2010.

2 Tanti B, Gurung L, Sarma HK & Buragohain AK, Ethnobotany of starter cultures used in alcohol fermentation by a few ethnic ethnic groups of Northeast India, *Indian J Tradit Knowle*, 9 (3) (2010) 463-466.

3 Tamang JP, Tamang N, Thapa S, Dewan S, Tamang BM, Yonzan H, Rai AK, Chettri R, Chakrabarty J & Kharel N, Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India, *Indian J Tradit Knowle*, 11 (1) (2012) 7-25.

4 Jeyaram K, Singh M, Capece A & Romano P, Molecular identification of yeast species associated with '*Hamei*"- a traditional starter used for rice wine production in Manipur, India, *Int J Food Microbiol*, 124 (2008) 115-125.

5 Thapa S & Tamang JP, Product characterization of *kodo ko jaanr*: fermented finger millet beverage of the Himalayas, *Food Microbiol,* 21 (2004) 617-622.

6 Tamang JP & Thapa S, Fermentation dynamics during production of bhaati jaanr, a traditional fermented rice beverage of the Eastern Himalayas, *Food Biotechnol*, 20 (2006) 251-261.

7 Tamang JP, Dewan S, Tamang B, Rai A, Schillinger U & Holzapfel WH, Lactic acid bacteria in *Hamei* and *Marcha* of North East India, *Indian J Microbiol,* 47 (2007) 119-125.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 29 May 2020
doi: 10.3389/fmicb.2020.00905

Diversity of Filamentous Fungi Isolated From Some Amylase and Alcohol-Producing Starters of India

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Filamentous fungi are important organisms in traditionally prepared amylase and alcohol-producing dry starters in India. We collected 40 diverse types of amylase and alcohol-producing starters from eight states in North East India viz. *marcha*, *thiat*, *humao*, *hamei*, *chowan*, *phut*, *dawdim*, and *khekhrii*. The average fungal population was 4.9×10^5 cfu/g with an average of pH 5.3 and 10.7%, respectively. In the present study, 131 fungal isolates were isolated and characterized based on macroscopic and microscopic characteristics and were grouped into 44 representative fungal strains. Based on results of morphological characteristics and ITS gene sequencing, 44 fungal strains were grouped into three phyla represented by Ascomycota (48%), Mucoromycota (38%), and Basidiomycota (14%). Taxonomical keys to species level was illustrated on the basis of morphological characteristics and ITS gene sequencing, aligned to the fungal database of NCBI GenBank, which showed seven genera with 16 species represented by *Mucor circinelloides* (20%), *Aspergillus sydowii* (11%), *Penicillium chrysogenum* (11%), *Bjerkandera adusta* (11%), *Penicillium citrinum* (7%), *Rhizopus oryzae* (7%), *Aspergillus niger* (5%), *Aspergillus flavus* (5%), *Mucor indicus* (5%) *Rhizopus microsporus* (5%), *Rhizopus delemar* (2%), *Aspergillus versicolor* (2%), *Penicillium oxalicum* (2%), *Penicillium polonicum* (2%), *Trametes hirsuta* (2%), and *Cladosporium parahalotolerans* (2%). The highest Shannon diversity index *H* was recorded in *marcha* of Sikkim (*H*: 1.74) and the lowest in *hamei* of Manipur (*H*: 0.69). Fungal species present in these amylolytic starters are morphologically, ecologically and phylogenetically diverse and showed high diversity within the community.

Keywords: filamentous molds, amylolytic starter, India, *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*

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Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Food Microbiology, a section of the journal Frontiers in Microbiology

Received: *01 December 2019*
Accepted: *16 April 2020*
Published: *29 May 2020*

Citation:
Anupma A and Tamang JP (2020) Diversity of Filamentous Fungi Isolated From Some Amylase and Alcohol-Producing Starters of India. Front. Microbiol. 11:905. doi: 10.3389/fmicb.2020.00905

INTRODUCTION

Drinking alcoholic beverages has a cultural connotation in India from the Indus Valley Civilization dating back to 8,000 years (Sarkar et al., 2016), mostly through fermentation (Singh et al., 2010) and distillation (Achaya, 1991). Traditionally malting, brewing (such as beer), and vinification (fermentation of grapes into wine) processes are unknown in Indian food culture. Instead, traditional alcoholic beverages are prepared either by natural fermentation of plants or cereals, or by using traditionally prepared dry starters in India (Tamang, 2010). Some ethnic people in India traditionally prepare amylase and alcohol-producing starters to ferment alcoholic beverages for

home consumption, which are known by different names in different languages spoken locally in regions such as *marcha* in Sikkim and Darjeeling hills, *thiat* in Meghalaya, *humao* in Assam, *hamei* in Manipur, *chowan* in Tripura, *phut* in Arunachal Pradesh, *dawdim* in Mizoramand *khekhrii* in Nagaland (Anupma et al., 2018), *dhehli, balam, maler, treh,* and *bakhar* of Himachal Pradesh and Uttarakhand (Thakur et al., 2015), and *ranu dabai/goti* of West Bengal, Odisha and Jharkhand (Ghosh et al., 2015). Traditional methods of the preparation of Indian starters are almost the same with some differences in use of starch-rich substrates such as rice or wheat or barley, and wrapping materials either in fern fronds or dry paddy-straw, or in fresh leaves of locally available wild plants (Shrivastava et al., 2012; Tamang et al., 2016). Soaked, dewatered, and ground cereal (rice/wheat/barley) flours are mixed with some wild plants, with a few spices such as sun-dried chilies or garlics and supplemented with 1–2% of previously prepared dry starters in powder forms ("back-slopping method" for sub-culturing the microbiota) to make thick doughs with addition of water. Thoroughly mixed dough mixtures are made into round or flat cakes of varying shapes and sizes, placed on fresh ferns or other plant leaves/dry paddy straws and allowed to ferment under semi-anaerobic conditions for 2–3 days at room temperature inside the room. After desirable fermentation, fermented doughs are then sun dried for 2–3 days to obtain dry starters which are exclusively used to ferment cereals into mild/strong alcoholic beverages (Tamang, 2010; Anupma et al., 2018). However, *khekhrii,* a dry starter from Nagaland in India is prepared by naturally fermenting sprouted-rice grains which are then dried in the sun to obtain dry starter granules to prepare an alcoholic beverage locally called *zutho*. Indian amylase and alcohol-producing starters are similar to starters from Asian countries such as *daqu* or *chiu* from China (Zheng et al., 2012), *benh* from Vietnam (Dung et al., 2007), *nuruk* from Korea (Jung et al., 2012), *ragi* from Indonesia (Roslan et al., 2018), *bubod* from the Philippines (Fronteras and Bullo, 2017), *loogpang* from Thailand (Daroonpunt et al., 2016) and *dombea* or *medombae* from Cambodia (Ly et al., 2018).

Several species of filamentous molds (Hesseltine et al., 1988; Yang et al., 2011; Lv et al., 2012a; Chen et al., 2014; Das et al., 2017); yeasts (Hesseltine and Kurtzman, 1990; Tamang and Sarkar, 1995; Tsuyoshi et al., 2005; Jeyaram et al., 2008, 2011; Thanh et al., 2008; Fronteras and Bullo, 2017; Sha et al., 2017, 2018, 2019), and bacteria (Hesseltine and Ray, 1988; Tamang et al., 2007; Sha et al., 2017; Roslan et al., 2018) are found to coexist in traditionally prepared dry starters as "micro-resources" which have been sub-cultured to preserve essential microbiota for alcohol production by Asian people for centuries (Tamang et al., 2020). Filamentous fungi present in traditional starters from Asia have several functionalities such as saccharification (Lee and Lee, 2002; Thapa and Tamang, 2004), liquefaction (Suesse et al., 2016), and ethanol production (Dung et al., 2007; Chen et al., 2014) to produce different types of low-alcoholic beverages and high-alcoholic distilled liquor. Filamentous molds are also responsible for the quality of alcoholic beverages including nutritional values and organoleptic properties such as flavor, taste, and color (Zhang et al., 2015; Tamang et al., 2016). Taxonomical identification of filamentous molds isolated from traditionally prepared dry starters from India have not been reported yet except from *marcha* (Tamang et al., 1988; Sha et al., 2017, 2019), *thiat* (Sha et al., 2017, 2019), *amou*, and *perok-kushi* (Das et al., 2017). *Mucor circinelloides*, *Rhizopus chinensis*, and *Rhizopus stolonifer* were reported earlier from *marcha* samples collected from Nepal, Darjeeling, and Sikkim (Tamang et al., 1988; Tamang and Sarkar, 1995; Thapa and Tamang, 2006; Sha et al., 2017, 2018), *Amylomyces rouxii* and *Rhizopus oryzae* from samples of *amou*, and *perok-kushi*, traditional starters of Assam (Das et al., 2017). Sha et al. (2017) reported fungal Phylum Ascomycota (98.6%) followed by Mucoromycota (1.4%), while in *marcha* samples only Phylum Ascomycota by high-through sequencing was reported. The present study aimed to identify the filamentous molds isolated from eight different types of traditionally prepared starters from North East India, viz. *marcha, thiat, humao, hamei, chowan, phut, dawdim*, and *khekhriii*, to species level by morphological and molecular identifications, and to profile their diversity within the fungal community.

MATERIALS AND METHODS

Sample Collection

A total of 40 samples of traditionally prepared dry starters viz *marcha* from Sikkim, *thiat* from Meghalaya, *humao* from Assam, *hamei* from Manipur, *chowan* from Tripura, *phut* from Arunachal Pradesh, *dawdim* from Mizoram, and *khekhrii* from Nagaland (**Table 1**) were collected directly from local markets and the homes of local producers in North East India (**Figure 1**) in pre-sterile containers. Dry starter samples were transported to the laboratory and stored in desiccators at room temperature as traditionally prepared dry starters have a shelf life of more than 1 year (Sha et al., 2018).

Analysis of pH and Moisture Content

The pH of homogenized samples was recorded by digital pH-meter (Orion 910003, Thermo Fisher Scientific, United States). The moisture content of the samples was estimated by a moisture analyzer (OHAUS/MB-45, United States).

Microbiological Analysis

Each dry sample starter was taken from the desiccator, then crushed coarsely by sterile spatula and 10 g of the crushed powered sample was homogenized with 90 mL of 0.85% physiological saline in a stomacher lab blender 40 (Seward, United Kingdom) for 2 min to obtain serial dilutions. One milliliter of each diluted sample (10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) was poured onto malt extract agar (M137, HiMedia, Mumbai, India) and potato dextrose agar (M096, HiMedia, Mumbai, India) with an addition of antibiotics (1% streptomycin) to suppress the growth of bacteria, and plates were then incubated under 28°C and observed for the appearance of colonies for up to 1 week. The colonies that appeared on plates were counted as a colony forming unit (cfu/g) on the dry weight of starters. Colonies were selected on the basis of macroscopic and microscopic characteristics. Selected filamentous molds were sub-cultured

TABLE 1 | Geographical locations, pH, moisture content, and fungal populations of dry starters from North East India.

Sample (n[a])	Region	Collection Site	Altitude (Meter)	Moisture content (%)	pH	cfu/g ($\times 10^5$)
Marcha (*n* = 8)	Sikkim	Gangtok	1637	11.6 (10.1 – 12.1)	5.2 (4.9 – 5.7)	5.0 (4.8 – 5.1)
		Basilakha	906			
		Pakyong	1341			
		Recabe	1072			
Thiat (*n* = 4)	Meghalaya	Shillong	1550	9.4 (8.7 – 10.0)	4.7 (4.5 – 5.0)	4.8 (4.5 – 5.1)
		Non-grem	1547			
Humao (*n* = 7)	Assam	Kokrajhar	49	9.7 (8.8 – 10.6)	4.9 (4.6 – 5.2)	4.6 (4.3 – 5.3)
		Jorhat	95			
		Sivsagar	93			
		Moran	100			
Hamei (*n* = 3)	Manipur	Kangchup	773	8.5 (8.0 – 9.6)	4.6 (4.1 – 5.4)	2.6 (2.5 – 3.2)
		Kakching	769			
		Phayeng	813			
Chowan (*n* = 4)	Tripura	Bangsul	116	9.1 (9.0 – 9.3)	5.6 (5.4 – 5.9)	3.1 (3.0 – 3.4)
		Dharmanagar	98			
Phut (*n* = 6)	Arunachal Pradesh	Doimukh	152	11.2 (11.4 – 11.8)	5.4 (5.5 – 5.7)	5.6 (4.9 – 5.9)
		Pasighat	155			
		Itanagar	361			
		Banderdewa	462			
		Nirjuli	151			
Dawdim (*n* = 3)	Mizoram	Saitual	438	13.7 (13.1 – 13.9)	6.2 (6.1 – 6.3)	7.4 (7.1 – 7.9)
Khekhrii (*n* = 5)	Nagaland	Kohima	1092	12.8 (12.3 – 13.1)	5.6 (5.5 – 5.9)	6.0 (5.7 – 6.8)

[a]*n = number of samples.*

on new plates and purified and stored on slants at 4°C for further studies.

Morphological and Physiological Identification

For each isolate, one- or three-point inoculations on petri plates containing ˜25 mL of media were applied. Fungal morphology was studied macroscopically by observing the colony features (surface color, reverse side color, shape, and diameter), and microscopically by observation of fruiting bodies using a stereomicroscope, and the vegetative and asexual stages were observed by a DE/Axio Imager A1 microscope (Carl Zeiss, Oberkochen, Germany) after staining freshly grown mycelia stained with cotton blue in MEA plates (Gaddeyya et al., 2012). Filamentous molds were identified on the basis of morphological features using the taxonomical keys described by Samson et al. (2004) and Pitt and Hocking (2009).

Genomic DNA Extraction

The genomic DNA was extracted from mold cultures following the methods of Umesha et al. (2016). Mycelial mass from the culture plate was scraped out by a sterile surgical blade and ground in a sterile mortar and pestle using 500 μL extraction buffer [100 mM Tris–HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% CTAB, and 0.2% 2 mercaptoethanol]. The mixture was transferred to a fresh 1.5 mL tube with addition of 4-μL RNase, vortexed and incubated for 60 min at 37°C, and kept in a water bath for 60 min at 55°C. 500 μL phenol: chloroform: isoamyl alcohol (25:24:1) was added to the solution, mixed thoroughly for 5 min, and then centrifuged at 14,000 rpm for 10 min. The aqueous clear phase was recovered and mixed with chloroform: isoamyl alcohol (24:1), centrifuged at 12,000 rpm for 5 min, and the aqueous phase was recovered, adding 0.8 volume of cold 7.5 M ammonium acetate and 0.54 volume of ice-cold isopropanol, and finally mixed well and stored overnight for precipitation of DNA in a deep freezer. The solution was centrifuged at 14,000 rpm for 3 min and precipitated with absolute ethanol to recover DNA. The DNA was then rinsed twice with 1 mL of 70% ethanol and resuspended in 100 μL of 1X TE [200 mM Tris–HCl (pH 8.0), 20 mM EDTA (pH 8.0)] buffer for further use and stored at −20°C. The quality of DNA was checked on agarose gel and the concentration was measured using a nanodrop spectrometer (ND-1000 spectrometer, NanoDrop Technologies, Willington, United States) (Kumbhare et al., 2015).

PCR Amplification

Polymerase chain reactions (PCR) of the internal transcribed spacer (ITS) region of filamentous molds was amplified using the primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Adekoya et al., 2017). PCR reactions were performed in 25 μL of PCR pre-master mix solution (Promega, United States). The amplification steps were followed: initial denaturation at 94°C for 5 min followed by 35 cycles consisting of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min, respectively; and a final extension at 72°C for 10 min in a Thermal Cycler (Applied biosystems-2720, United States). The PCR products were verified by electrophoresis on 1.0% agarose gel containing 0.7 mg/mL of

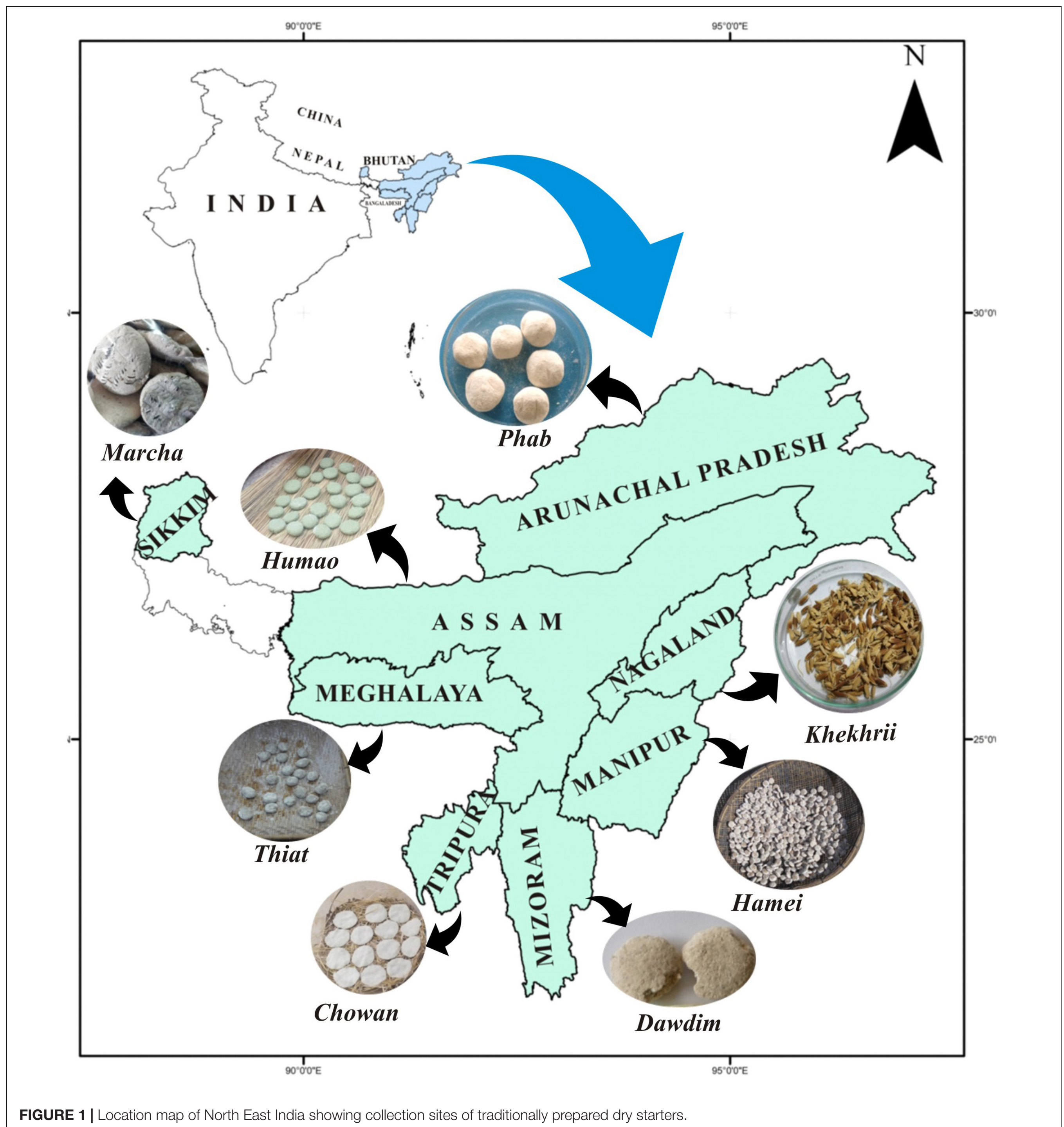

FIGURE 1 | Location map of North East India showing collection sites of traditionally prepared dry starters.

ethidium bromide and visualized under UV light (Gel doc 1000, Bio-Rad, 97-0186-02, United States). Approximate size of amplicons was determined using standard molecular markers (Himedia-100 bp DNA ladder, Mumbai, India).

Purification of the PCR Amplicons

The amplified PCR products were purified using PEG (polyethylene glycol)-NaCl (sodium chloride) and precipitation solution (20% w/v of PEG, 2.5 M NaCl) with the addition of 0.6 volumes of 20% PEG-NaCl to the final volume of the PCR products (Schmitz and Riesner, 2006). The mixture was centrifuged at 12,000 rpm for 30 min, incubated at 37°C for 30 min, the aqueous solution was discarded, and the pellet was washed twice with 1 mL ice cold 70% freshly prepared ethanol (70%). The collected pellet was then air dried prior to elution in 20 μL of nuclease-free

water, and finally, the purified product was loaded in 1% agarose gel.

ITS Sequencing

PCR-amplified products had been sequenced in a forward and reverse direction using ITS1 primer (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′), respectively, as per the method described by Martin and Rygiewicz (2005). The PCR reaction was carried out in 50 μL reaction volume containing 2.0 mM MgCl2, 0.2 μM each primer, 0.2 mM dNTP, 0.5 mg $[mL]^{-1}$ bovine serum albumin (BSA) and 0.04 U $[\mu L]^{-1}$ tTaq DNA polymerase on a thermal cycler equipped with a heated lid. The thermal program included initial denaturation, enzyme activation at 95°C (6–10 min) followed by 35 cycles to complete the step [95°C (1 min), 40°C (2 min) and 72°C (1 min)] and one cycle at 72°C (10 min). The amplified products were sequenced by an automated DNA Analyzer (ABI 3730XL Capillary Sequencers, Applied Biosystems, Foster City, CA, United States). These high-quality, double-stranded sequence data were analyzed with the help of the BLASTn program and multiple sequence alignment.

Bioinformatics

The qualities of the raw sequences were checked by Sequence Scanner version 1.0 (Applied Biosystems, Foster City, CA, United States) and were edited using software ChromasPro version 1.34. Sequences were compared with sequence entries in the GenBank of NCBI (National Center for Biotechnology Information)[1] using the Basic Local Alignment Search Tool for nucleotides (BLASTn) on the NCBI website (Pinto et al., 2012). For phylogenetic analysis, the available sequence of similar related organisms was retrieved in FASTA format and aligned using the clustal-W. Sequence alignment and a phylogenetic tree were constructed using MEGA7.0 software by Neighbor-Joining methods using 1000-bootstrap replicates (Lutzoni et al., 2004).

Statistical Analysis

Percentages of frequency and relative density of fungal species in samples were determined as per the method described by Doi et al. (2018). Frequency (%) was calculated by the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

Relative Density (%) was calculated by the equation:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats studied}} \times 100$$

Diversity indexes of filamentous molds in samples were calculated by species richness (R), Shannon's diversity index (H), and species evenness (E) (Panda et al., 2010) using PAST (Paleontological STatistics) software version 3.26 (Hammer et al., 2001).

Nucleotide Sequence Accession Numbers

The sequences obtained in this study were deposited at the GenBank-NCBI database under accession numbers: MK396469–MK396484, MK396486–MK396500, MK778442–MK778449, and MK796041–MK796045.

RESULTS

Microbial Load, pH, and Moisture

The microbial load of filamentous molds in 40 samples of traditionally prepared dry starters collected from different regions of North East India were 2.5 to 7.9 × 10^5 cfu/g (**Table 1**). The pH and moisture contents of all samples analyzed were pH 4.1–6.3 and 8.0–13.9%, respectively (**Table 1**).

Morphological Characterization

We isolated 131 total fungal isolates from 40 different samples of traditionally prepared dry starters (*marcha*, *thiat*, *humao*, *hamei*, *chowan*, *phut*, *dawdim*, and *khekhrii*) collected from eight states of North East India (**Table 1**). Based on the morphological characteristics (such as color, texture, size, and appearance of colony), microscopic characteristics (sporangia, sporangiospores, chlamydospores, conidia, conidiophore, and rhizoid structure), 44 representative fungal isolates were grouped (seven isolates from *marcha*, five from *thiat*, six from *humao*, two from *hamei*, five from *chowan*, six from *phut,* six from *dawdim*, and seven from *khekhrii*). *Mucor*, *Rhizopus*, *Aspergillus, Penicillium,* and *Cladosporium* and a few unidentified basidiomycetes fungi were tentatively identified on the basis of detailed morphological characters using the taxonomical keys described by Samson et al. (2004) and Pitt and Hocking (2009) (**Supplementary Table S1**).

Molecular Identification of Fungal Isolates

Genomic DNA of each isolate of 44 representative fungal strains was extracted and PCR products were prepared for identification by ITS gene sequencing. DNA sequences of fungal isolates were assigned by comparison with those available in the GenBank of NCBI database using the ITS gene sequence (ITS1 and ITS4) based on the Basic Local Alignment Search Tool (BLAST) 2.0 program (Raja et al., 2017). The phylogenetic trees of nucleotide sequences of the 44 fungal isolates from the samples were constructed using the Neighbor-joining method with 1000 replicates bootstrap values (**Figure 2**). ITS gene sequencing results showed three fungal phyla represented by Ascomycota (48%), Mucoromycota (38%), and Basidiomycota (14%) (**Figure 3**). Distribution percentage of the phyla in the starter showed the highest percentage of Ascomycota (86%) in

[1] http://www.ncbi.nlm.nih.gov/Blast.cgi

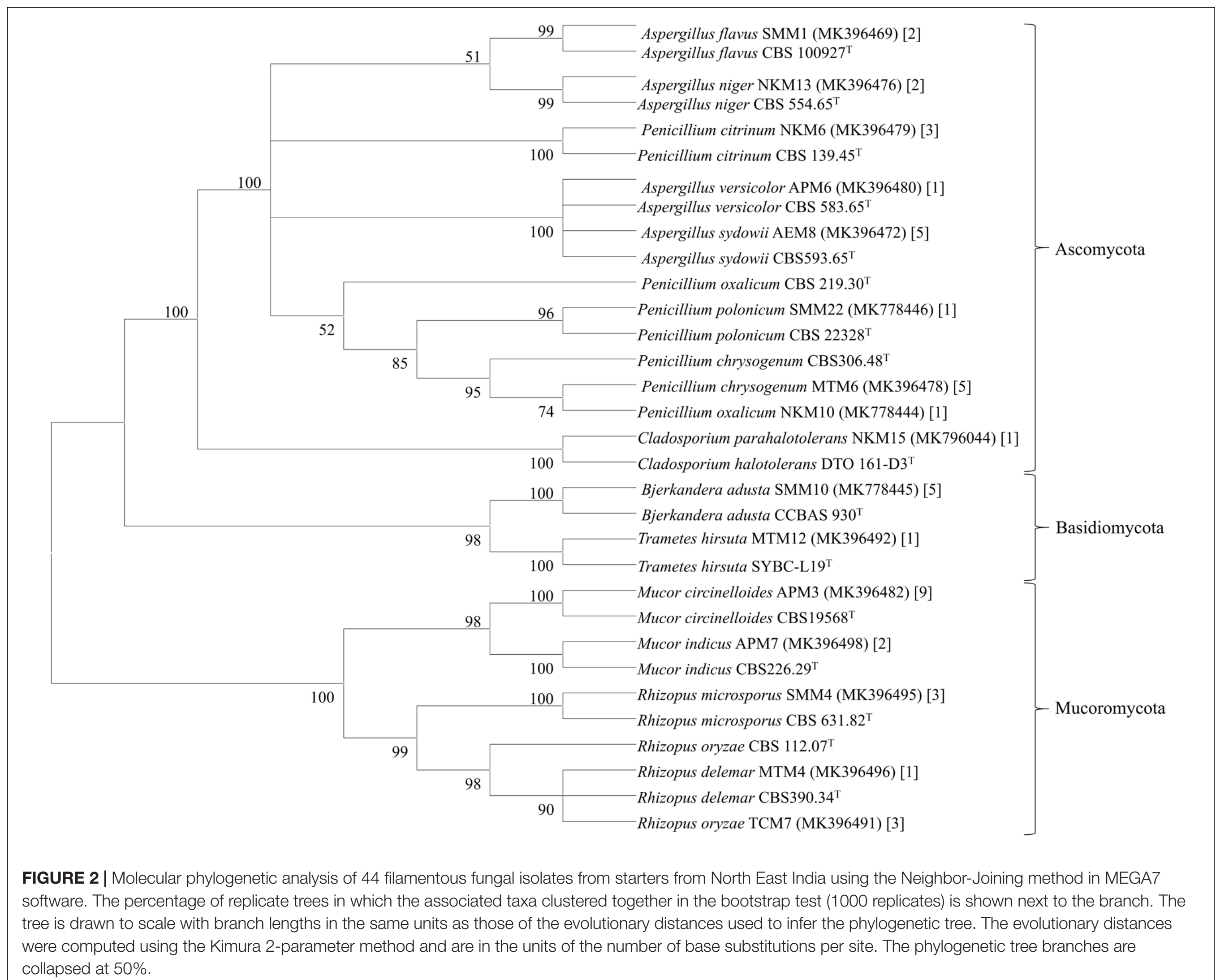

FIGURE 2 | Molecular phylogenetic analysis of 44 filamentous fungal isolates from starters from North East India using the Neighbor-Joining method in MEGA7 software. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branch. The tree is drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The phylogenetic tree branches are collapsed at 50%.

khekhrii, Mucoromycota (60%) in *dawdim*, and Basidiomycota (20%) in *chowan, dawdim*, and *thiat*, respectively. Phyla Ascomycota and Mucoromycota were present in all starters, whereas Basidiomycota was present only in *marcha*, *thiat, chowan*, and *dawdim.*

Based on results of morphological characteristics and ITS gene sequencing, 44 representative strains of filamentous molds isolated from traditionally prepared dry starters from India were grouped into seven genera with 16 species, which were represented by *Mucor circinelloides* (20%), *Aspergillus sydowii* (11%), *Penicillium chrysogenum* (11%), *Bjerkandera adusta* (11%), *Penicillium citrinum* (7%), *Rhizopus oryzae* (7%), *Aspergillus niger* (5%), *Aspergillus flavus* (5%), *Mucor indicus* (5%) *Rhizopus microsporus* (5%), *Rhizopus delemera* (2%), *Aspergillus versicolor* (2%), *Penicillium oxalicum* (2%), *Penicillium polonicum* (2%), *Trametes hirsuta* (2%), and *Cladosporium parahalotolerans* (2%) (**Table 2** and **Figure 4**). Interestingly we detected few basidiomycetes fungi represented by *Bjerkandera adusta* and *Trametes hirsuta* in *marcha, thiat, chowan* and *dawdim* samples. Colony morphology and microscopic images of 16 species of seven genera of filamentous molds isolated from dry starters from India were illustrated for fungal taxonomy (**Figure 5**).

Frequency and density of fungal species in samples showed that *Aspergillus niger* was colonized with *khekhrii;* a species from the *Mucor circinelloides* complex was observed with a high dominance in samples, whereas *Trametes hirsuta* was less diversified and observed only in *thiat* samples (**Table 3**).

Diversity indexes of filamentous molds of dry starters were characterized by species richness (R), Shannon's diversity index (H), and species evenness (E) (**Table 3**). The Shannon diversity index *H* was recorded as the highest in *marcha* from Sikkim (*H*: 1.74) and the lowest in *hamei* from Manipur (*H*: 0.69). Species Evenness (E) values were 0.97 in *marcha* followed by *humao* from Assam and *phut* from Arunachal Pradesh. The Species Richness (R), values were recorded highest in *marcha* and *khekhrii* samples (**Table 3**).

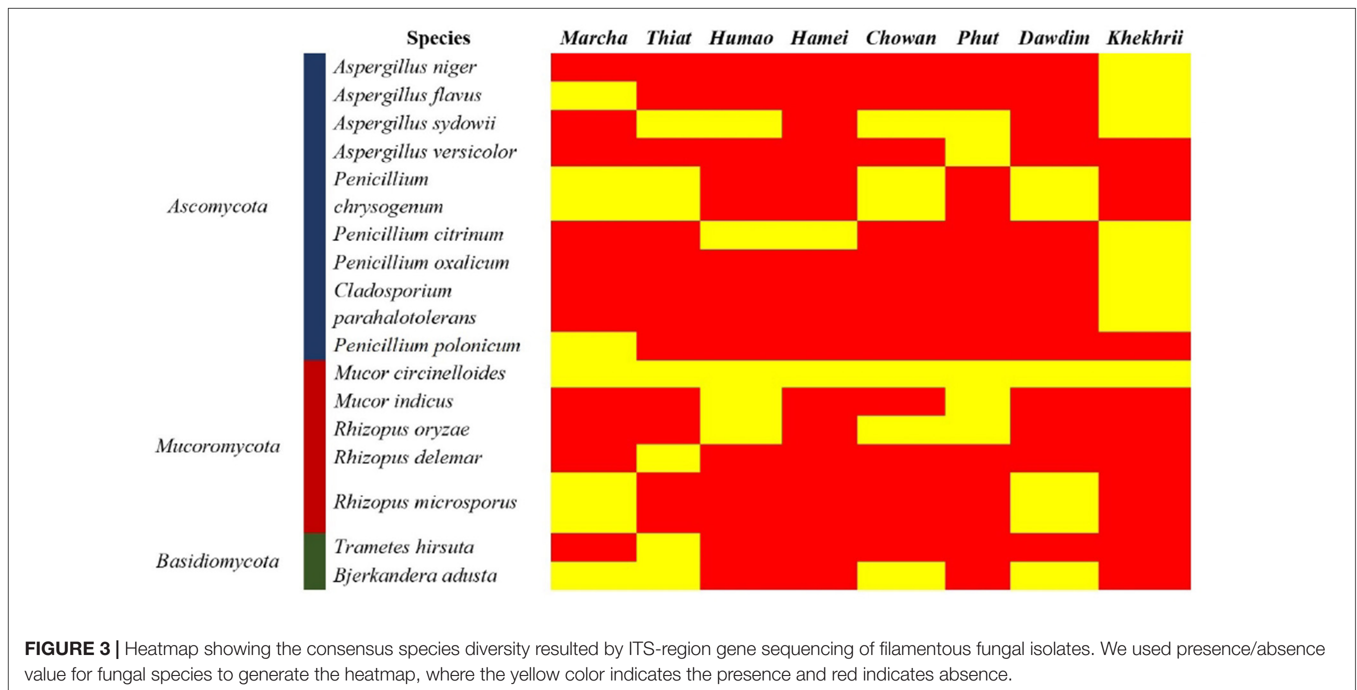

FIGURE 3 | Heatmap showing the consensus species diversity resulted by ITS-region gene sequencing of filamentous fungal isolates. We used presence/absence value for fungal species to generate the heatmap, where the yellow color indicates the presence and red indicates absence.

DISCUSSION

Drinking of cereal-based mild to strong alcoholic beverages produced by traditionally prepared amylase and alcohol-producing starters has been a traditional food culture of the ethnic people from the North East states of India for centuries. Traditionally prepared dry starters have consortia of co-existed microbiota containing filamentous molds, yeasts, and bacteria and are crudely sub-cultured through a "back-slopping" process by traditional starter-makers (Hesseltine et al., 1988; Tamang and Sarkar, 1995; Tamang et al., 2007; Sha et al., 2018, 2019), for alcohol production by the Indian people. The pH of traditionally prepared dry starters from India were slightly acidic in nature, perhaps due to accumulation of metabolic organic acids (Ma et al., 2019). Moreover, low pH is favorable for the growth of mycelial fungi (Abubakar et al., 2013). Low content of moisture in starter cultures is due to the sun-drying process during the traditional method of preparation practiced by the ethnic people of India, which may increase the shelf life of the starter for a year or more at room temperature (Tsuyoshi et al., 2005; Tamang, 2010).

Some traditionally prepared starters from North East India have been microbiologically analyzed in earlier works and several species of yeasts (Tsuyoshi et al., 2005; Jeyaram et al., 2008, 2011; Sha et al., 2017, 2018, 2019) and bacteria (Tamang et al., 2007; Pradhan and Tamang, 2019) were reported. However, detailed taxonomical studies of filamentous molds isolated from traditionally prepared dry starters from North East India have not been reported yet, except for *marcha* (Tamang et al., 1988; Tamang and Sarkar, 1995; Sha et al., 2017, 2019), *thiat* (Sha et al., 2017, 2019), *amou, perok-kushi* (Das et al., 2017). Hence, we studied the taxonomy and diversity of filamentous fungi associated with traditionally prepared dry starter cultures from North East India viz., *marcha* from Sikkim, *thiat* from Meghalaya, *humao* from Assam, *hamei* from Manipur, *chowan* from Tripura, *phut* from Arunachal Pradesh, *dawdim* from Mizoram, and *khekhrii* from Nagaland based on morphological characters and molecular identifications. The average fungal population in traditionally prepared dry starters from North East India was 10^5 cfu/g, which was in accordance with earlier reports on fungal populations in *marcha* of Sikkim, and the Darjeeling hills in India (Tamang et al., 1988; Tamang and Sarkar, 1995). No such data on fungal population in other starters of India are available except for *marcha*. In the present study, we first isolated and characterized 131 fungal isolates from 40 different starters from North East India based on macroscopic and microscopic characteristics and grouped them into 44 representative fungal strains. Morphological examination and identification of fungi are useful for identification up to the family or genus level (Alsohaili and Bani-Hasan, 2018). However, morphological-based identification is not adequate to identify the fungi up to species level (Lutzoni et al., 2004). The sequence-based identification tool is widely applied to confirm the exact identify of the fungal species (Romanelli et al., 2010; Xu, 2016).

We applied polymerase chain reactions (PCR) of the internal transcribed spacer (ITS) region of 44 strains of filamentous fungi isolated from starters from North East India using the primers ITS1 and ITS4 and grouped into three phyla represented by Ascomycota (48%), Mucoromycota (38%), and Basidiomycota (14%). A similar type of phylum distribution was also reported earlier in a *nuruk* dry starter from Korea (Carroll et al., 2017) and *daqu* from China (Shoubao et al., 2019). Seven genera with 16 species of filamentous fungi, isolated from Indian amylase

TABLE 2 | Molecular identification of filamentous molds isolated from starters from North East India by ITS gene sequence (ITS1 and ITS4) based on BLAST.

Product	Isolate code	Identity	GenBank accession number	Size in base pair (arbitrary primers)
Marcha	SMM-1	*Aspergillus flavus*	MK396469	519
	SMM-3	*Mucor circinelloides*	MK396489	642
	SMM-4	*Rhizopus microsporus*	MK396495	703
	SMM-10	*Bjerkandera adusta*	MK778445	675
	SMM-16	*Penicillium chrysogenum*	MK396477	577
	SMM-22	*Penicillium polonicum*	MK778446	582
	SMM-35	*Penicillium chrysogenum*	MK778447	552
Thiat	MTM-1	*Mucor circinelloides*	MK396487	636
	MTM-4	*Rhizopus delemar*	MK396496	768
	MTM-6	*Penicillium chrysogenum*	MK396478	583
	MTM-12	*Trametes hirsuta*	MK396492	637
	MTM-16	*Bjerkandera adusta*	MK396500	651
Humao	AEM-1	*Penicillium citrinum*	MK396481	437
	AEM-3	*Rhizopus oryzae*	MK396483	613
	AEM-4	*Mucor circinelloides*	MK396484	648
	AEM-8	*Aspergillus sydowii*	MK396472	467
	AXM-1	*Aspergillus sydowii*	MK396475	546
	AMM-3	*Mucor indicus*	MK778442	565
Hamei	MHM-1	*Mucor circinelloides*	MK796043	601
	MHM-15	*Penicillium citrinum*	MK796042	469
Chowan	TCM-1	*Bjerkandera adusta*	MK396494	520
	TCM-4	*Mucor circinelloides*	MK778449	636
	TCM-7	*Rhizopus oryzae*	MK396491	637
	TCM-9	*Aspergillus sydowii*	MK796041	541
	TCM-12	*Penicillium chrysogenum*	MK778448	541
Phut	APM-1	*Aspergillus sydowii*	MK396473	577
	APM-3	*Mucor circinelloides*	MK396482	645
	APM-6	*Aspergillus versicolor*	MK396480	417
	APM-7	*Mucor indicus*	MK396498	627
	APM-12	*Rhizopus oryzae*	MK396490	621
	APM-15	*Aspergillus sydowii*	MK396474	574
Dawdim	MDM-1	*Mucor circinelloides*	MK396497	645
	MDM-10	*Bjerkandera adusta*	MK396493	569
	MDM-11	*Rhizopus microsporus*	MK396488	696
	MDM-14	*Mucor circinelloides*	MK396486	641
	MDM-16	*Bjerkandera adusta*	MK396499	680
	MDM-18	*Penicillium chrysogenum*	MK778443	554
Khekhrii	NKM-1	*Mucor circinelloides*	MK796045	490
	NKM-6	*Penicillium citrinum*	MK396479	519
	NKM-7	*Aspergillus flavus*	MK396470	519
	NKM-8	*Aspergillus niger*	MK396471	551
	NKM-10	*Penicillium oxalicum*	MK778444	581
	NKM-13	*Aspergillus niger*	MK396476	602
	NKM-15	*Cladosporium parahalotolerans*	MK796044	546

and alcohol-producing starters, were identified based on the morphological and microscopic characteristics, and molecular identification which were represented by *Aspergillus flavus, A. niger*, *A. sydowii*, *A. versicolor*, *Bjerkandera adusta*, *Cladosporium parahalotolerans*, *Mucor circinelloides*, *M. indicus*, *Penicillium chrysogenum*, *P. citrinum*, *P. oxalicum*, *P. polonicum, Rhizopus delemar, R. microsporus, R. oryzae*, and *Trametes hirsuta*. Illustration of taxonomical keys based on morphological and molecular identification is more accurate and reliable in fungal taxonomy (Xing et al., 2018). Our earlier findings of *Rhizopus oryzae* and species from the *Mucor circinelloides* complex in traditionally prepared starters of North East India by PCR-DGGE method (Sha et al., 2018) supported the present study. Hesseltine and Kurtzman (1990) reported species from the *M. circinelloides* complex in *bubod* from the Philippines. Species from the *M. circinelloides* complex*, M. indicus, Rhizopus oryzae,* and *R. microsporus* were reported i*n benh men* from Vietnam (Dung et al., 2007; Thanh et al., 2008). In *marcha* and *khekhrii*

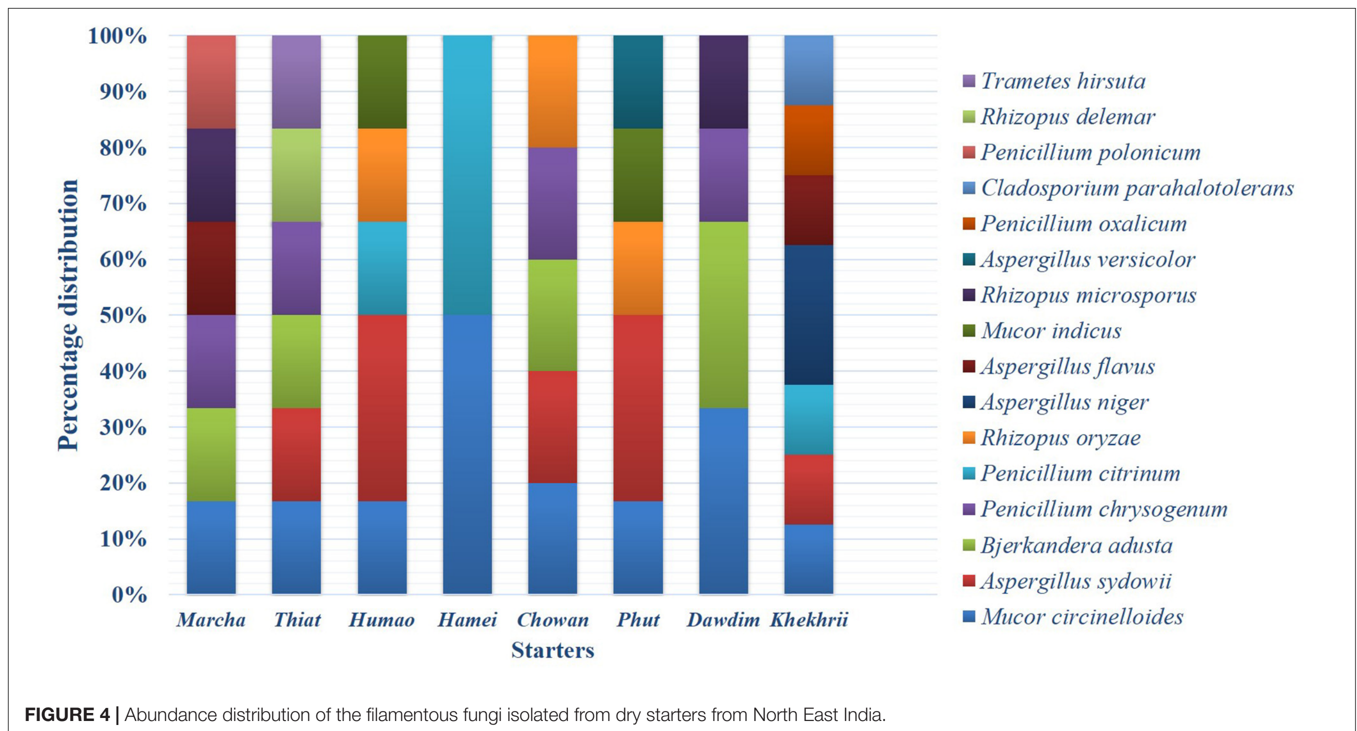

FIGURE 4 | Abundance distribution of the filamentous fungi isolated from dry starters from North East India.

we detected *Aspergillus flavus*, which was also reported in *mana*, an amylolytic starter from Nepal (Nikkuni et al., 1996).

Aspergillus belonging to order Eurotials is a phenotypically polythetic genus and is widely distributed in the environment (Tsang et al., 2018). Samson et al. (2014) proposed phylogenic identification of *Aspergillus* with ITS sequence data, and calmodulin as a secondary identification marker, according to the decision of the International Commission of *Penicillium* and *Aspergillus*[2]. Application of ITS with β-tubulin sequences for identification of *Aspergillus* species has also been reported by Zulkifli and Zakaria (2017). However, in this study we have applied both ITS sequence and morphological characteristics, such as the conidiophore with straight ending in a large vesicle from where primary and secondary sterigmata arise bearing conidia in chains, for identification of species of *Aspergillus*. *Aspergillus niger* and *A. flavus* cannot be distinguished only by their ITS sequences, the morphological characters are also essential in species identification (Zulkifli and Zakaria, 2017). We identified genus *Aspergillus* with four species in dry starter samples from India which included *A. niger*, *A. flavus*, *A. sydowii,* and *A. versicolor*. Among *Aspergillus A. flavus, A. niger* and *A. sydowii* were most prevalent in food samples due to their sporulating ability in the environment (Adekoya et al., 2017). *Aspergillus* is a dominant fungal genus in *daqu* from China (Ji et al., 2018), and may contribute to the saccharification process (Wang et al., 2019). We detected two strains of *Aspergillus flavus* in a *marcha* sample from Sikkim (*Aspergillus flavus* SMM-1) and in a *khekhrii* sample from Nagaland (*A. flavus* NKM-7). Though the distribution percentage was only 5%, the presence of *A. flavus* in samples of *marcha* and *khekhrii* is alarming. *A. flavus* is a saprotrophic with cosmopolitan distribution (Ramírez-Camejo et al., 2012), which produces aflatoxin (Saori and Keller, 2011; Priyanka et al., 2012; Mudili et al., 2014). Probable sources of *A. flavus* in starters may be from contaminated rice grains (Lai et al., 2015) since rice is the main base substrates for the preparation of starters for the production of alcohol. Moreover starter-makers commonly use low-quality, old-stocked and discarded rice grains for preparation of starters. However due to co-existence of other species of filamentous molds, yeasts and lactic acid bacteria in traditionally prepared starters may antagonize against *A. flavus* in *marcha* and *khekhrii,* which may reduce aflatoxin production in the sample (Karlovsky et al., 2016; Adebo et al., 2019). Lactic acid bacteria isolated from *marcha* showed an antagonistic property (Tamang et al., 2007), similarly, some bacteria have antifungal activity against aflatoxin-producing *A. flavus* (Shakeel et al., 2018). *Rhizopus* spp. from *tempeh,* a fermented soybean food from Indonesia, were reported for detoxification of alfatoxins (Nakazato et al., 1990). *A. sydowii* present in samples *humao*, *phut* and *chowan*, is an industrially important filamentous mold, which produces monosaccharides and indole alkaloids (Zhou et al., 2018). None of the amylolytic starters of North East India showed the presence of *A. versicolor* except in *phut* samples from Arunachal Pradesh. *A. versicolor* is a slow-growing filamentous fungus commonly found in/on damp indoor environments (Samson et al., 2004), foods, and feeds (Jurjevic et al., 2012), and produces toxic metabolites (Piontek et al., 2016). Contamination of *A. versicolor* in *phut* samples might be from the damp room where preparation of *phut* is often practiced by starter-producers in Arunachal Pradesh.

[2]www.aspergilluspenicillium.org

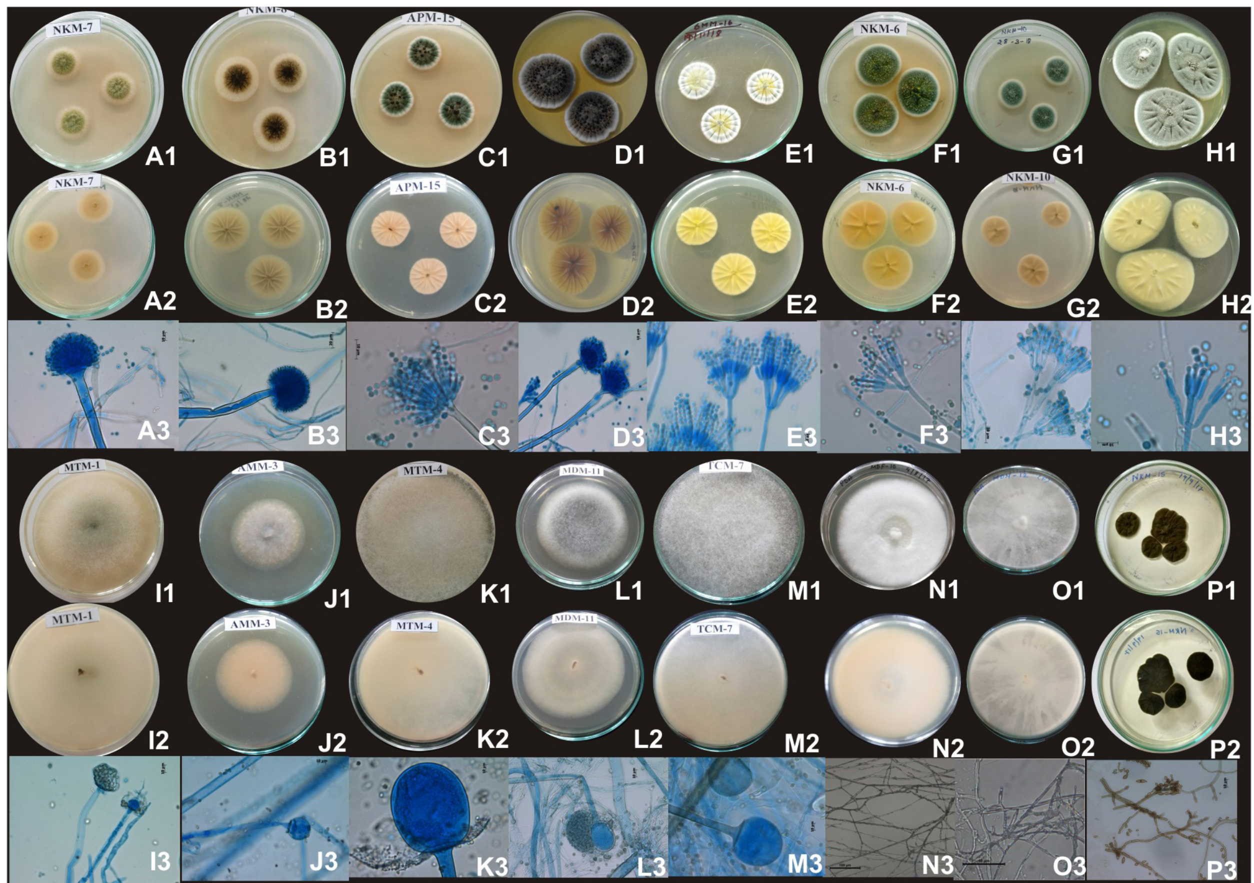

FIGURE 5 | Images of colony morphology and microscopic features of filamentous molds that grew on MEA media: *Aspergillus flavus* colonies top **(A1)**, reverse **(A2)**, Conidiophores **(A3)**; *Aspergillus niger* colonies top **(B1)**, reverse **(B2)**, mature conidia globose conidial head contain conidia **(B3)**; *Aspergillus sydowii* colonies top **(C1)**, reverse **(C2)**, mature conidiophore with vesicle bearing conidiogenous metulae and phailides (biserate) **(C3)**; *Aspergillus versicolor* colonies top **(D1)**, reverse **(D2)**, conidial heads supported vesicles with which are biseriate with metulae about the same size of philiades **(D3)**; *Penicillium chrysogenum* colonies top **(E1)**, reverse **(E2)**, smooth-walled conidiophores stipes (150–280 μm) and biverticillate **(E3)**; *Penicillium citrinum* colonies top **(F1)**, reverse **(F2)**, conidiophores stipes (150–280 μm) and biverticillate, phialides ampuliform (flask-shaped) **(F3)**; *Penicillium oxalicum* colonies top **(G1)**, reverse **(G2)**, mature conidiophores monoverticillate, or biverticillate and asymmetrical, phialides were cylindrical; *Penicillium polonicum* colonies top **(H1)**, reverse **(H2)**, conidiophore were terverticillate, phialides **(H)**; *Mucor circinelloides* colonies top **(I1)**, reverse **(I2)**, mature sporangiosphores contain sporangiospores **(I3)**; *Mucor indicus* colonies top **(J1)**, reverse **(J2)**, mature sporangiosphores contain sporangiospores **(J3)**; *Rhizopus delemar* colonies top **(K1)**, reverse **(K2)**, globose sporangium **(K3)**; *Rhizopus oryzae* colonies top **(L1)**, reverse **(L2)**, sporangiophores were usually straight, mostly 10∼20 μm **(L3)**; *Rhizopus microsporus* colonies top **(M1)**, reverse **(M2)**, sporangia globose, smooth and released spore **(M3)**; *Trametes hirsuta* colonies top **(N1)**, reverse **(N2)**, hyphal structure **(N3)**; *Bjerkandera adusta* colonies top **(O1)**, reverse **(O2)**, dichotomously branched hyphae **(O3)**; *Cladosporium parahalotolerans* colonies top **(P1)**, reverse **(P2)**, conidiophores and conidial chain **(P3)**.

Mucor circinelloides was found to be the most dominant fungus in dry starter cultures from North East India. *M. circinelloides* has a sub-globose sporangiospore with a sympodial branching pattern. Using the ITS sequencing tool, it is difficult to distinguish among the different species of the *Mucor circinelloides* complex (MCC) which include *M. circinelloides, M. griseocyanus, M. janssenii, M. lusitanicus, M. ramosissimus, M. variicolumellatus*, and *M. velutinosus* (Wagner et al., 2019). We therefore used species from the *Mucor circinelloides* complex. *Mucor circinelloides* contributes in saccharification and liquefaction of cereal during fermentation of *kodo ko jaanr*, an alcoholic product of Sikkim fermented by starter *marcha* (Thapa and Tamang, 2004; Tamang and Thapa, 2006). *M. circinelloides* is an oleaginous fungus (Qiao et al., 2018) which produces lipids (Wei et al., 2013), cellulose degrading enzymes (Huang et al., 2014), and has several functional properties including antioxidants (Hameed et al., 2017). Phylum Mucoromycota does not produce mycotoxins, however, some species that belong to this *M. circincelloides* forma *circinelloides* group has been described to be putatively responsible for human illnesses after consumption of mold-contaminated yogurt (Lee et al., 2014) although its involvement was not clearly proven. *M. circinelloides* was also reported earlier in *marcha* samples (Tamang et al., 1988; Tamang and Sarkar, 1995). *M. indicus*, isolated from *humao* from Assam and *phut* from Arunachal Pradesh, is a dimorphic and ethanolic fungus which is able to produce ethanol from glucose, mannose, fructose and galactose (Karimi and Zamani, 2013) and oil, protein, and glucosamine (Sharifyazd and Karimi, 2017).

TABLE 3 | Frequency, density, and diversity indices of filamentous molds observed in dry starters from North East India.

Filamentous molds	***Marcha***		***Thiat***		***Humao***		***Hamei***		***Chowan***		***Phut***		***Dawadim***		***Khekhari***	
	%															
	Fr	**RD**	**Fr**	**RD**	**Fr**	**RD**	**Fr**	**RD**	**Fr**	**RD**	**Fr**	**RD**	**Fr**	**RD**	**Fr**	**RD**
Aspergillus niger	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0.25
Aspergillus flavus	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0	12.5	0.12
Aspergillus sydowii	0	0	16.6	0.16	33.3	0.33	0	0	20	0.2	33.3	0.33	0	0	12.5	0.12
Aspergillus versicolor	0	0		0	0	0	0	0		0	16.6	0.16	0	0	0	0
Penicillium chrysogenum	16.6	0.16	16.6	0.16	0	0	0	0	20	0.2	0	0	16.6	0.16	0	0
Penicillium citrinum	0	0	0	0	16.6	0.16	50	0.5	0	0	0	0	0	0	12.5	0.12
Penicillium oxalicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5	0.12
Cladosporium parahalotolerans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5	0.12
Penicillium polonicum	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mucor circinelloides	16.6	0.16	16.6	0.16	16.6	0.16	50	0.5	20	0.2	16.6	0.16	33.3	0.33	12.5	0.12
Mucor indicus	0	0		0	16.6	0.16	0	0	0	0	16.6	0.16	0	0	0	0
Rhizopus oryzae	0	0		0	16.6	0.16	0	0	20	0.2	16.6	0.16	0	0	0	0
Rhizopus delemar	0	0	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus microsporus	16.6	0.16667		0	0	0	0	0	0	0	0	0	16.6	0.16	0	0
Trametes hirsuta	0	0	16.6	0.16	0	0	0	0	0	0	0	0	0	0	0	0
Bjerkandera adusta	16.6	0.16667	16.6	0.16	0	0	0	0	20	0.2	0	0	33.3	0.33	0	0
DIVERSITY INDICES																
Species richness (R)	6		5		5		2		5		5		4		6	
Shannon's diversity index (H)	1.74		1.6		1.56		0.69		1.6		1.56		1.32		1.46	
Species evenness (E)	0.97		1		0.96		1		1		0.96		0.95		0.82	

Fr, Frequency of fungal species; RD, Relative density of fungal species in samples.

Phylogenetic and phylogenomic approaches show that genus *Rhizopus* has three major clades viz. *R. microsporus with* its sister taxon *R. stolonifer*, *R. arrhizus*, and *R. delemar* (Gryganskyi et al., 2018). *Rhizopus oryzae,* commonly inhabits soils, animal excrement, and rotting vegetables (Ghosh and Ray, 2011), and is very similar to *Rhizopus stolonifer*, except for its smaller sporangia with air-dispersed sporangiospores (Pitt and Hocking, 2009). *R. oryzae* and *R. microsporus* are detected in *yao qu* from China and *banh men* from Vietnam, which are strong amylase producers (Dung et al., 2007; Thanh et al., 2008; Lv et al., 2012b). *R. oryzae* is considered as a GRAS filamentous fungus (Londoño-Hernández et al., 2017), which is commonly used for production of some Asian fermented foods (Tamang et al., 2016). *Rhizopus microsporus* is the major fungus in *tempe*, a fermented soybean food from Indonesia (Hartanti et al., 2015). *R. delemar* was found in the *thiat* sample only, which naturally accumulates fumaric acid with a fruity taste (Odoni et al., 2017), and it probably imparts taste and flavor in *kiad,* an alcoholic product fermented by the starter *thiat. R. delemar* has also been reported in *xajpitha,* starter from Assam in India (Bora et al., 2016). Presence of *Rhizopus* spp. in starters from North East India may contribute functionalities in end products during acholic fermentation.

Penicillium chrysogenum was found in only four types of starters viz. *marcha* (Sikkim), *thiat* (Meghalaya), *chowan* (Tripura), and *dowdim* (Mizoram). The probable entry of *P. chrysogenum* during traditional preparation may be from damp and moist rooms where preparation for such starters is usually done, since *P. chrysogenum* is also found in damp buildings (Andersen et al., 2011). Due to the ability of *P. chrysogenum* to produce antibiotics, mostly penicillin (Bajaj et al., 2014), its presence in starters may have an antagonist property in the end product. *P. citrinum* was recovered in samples of *humao, hamei* and *khekhrii*, probably from indoor environments (Samson et al., 2004). *P. oxalicum* was found in samples of *khekhrii* (Nagaland) and *P. polonicum* in *marcha* samples. *P. oxalicum* produces various enzymes and natural products (Li et al., 2016). *P. polonicum* has also been reported in fermented black table olives (Bavaro et al., 2017).

It is interesting to note that we detected Basidiomycetous fungi represented by *Bjerkandera adusta* in samples of *marcha, thiat, dawdim,* and *chowan*, and also *Trametes hirsuta* in *thiat* samples. *Bjerkandera adusta* and *Trametes hirsuta* are wood decaying white-rot fungi (Rosales et al., 2005; Horisawa et al., 2019). *B. adusta* grows on a natural cellulosic substrate, imparts a refreshing aroma (Zhang et al., 2015), contributes to saccharification (Quiroz-Castañeda et al., 2009), and produces ethanol (Horisawa et al., 2019). *Trametes hirsuta* is lignin-degrading fungus due its ability to synthesize laccase (Cilerdzic et al., 2011). Traditional methods of preparation of these amylolytic starter cultures require locally grown wild herbs and spices used as ingredients by local starter-makers (Anupma et al., 2018). We assume that during collection of wild herbs from forest grounds, people might have collected whole wild plants *in situ*, where wood-rooting fungi have been reported in forests of North East India (Chuzho et al., 2017). There is no practice of filtering and cleaning of collected wild plants during starter preparation, hence chances for contamination of these basidiomycetous fungi may be possible during preparation. *B. adusta* and *T. hirsuta* were not reported earlier in any starter culture or in any fermented food.

Cladosporium parahalotolerance was found only in samples of *khekhrii*. *C. parahalotolerance* mostly occurred in plant debris, foods, and indoors (Bensch et al., 2012). Source of *Cladosporium* in *khekhrii* might be from wild herbs used as ingredients during traditional preparation of *khekhrii* in Nagaland. Species of *Bjerkandera, Trametes,* and *Cladosporium* have not been reported in any fermented foods elsewhere.

Diversity indexes determine the phylogenetic relations within different fungal species in a community (Fernandes et al., 2015). We calculated diversity indexes of fungal community present in starters of North East India by Shannon's diversity index (H), species evenness (E), and species richness (R). Shannon diversity index *H* for evaluating fungal diversity was recorded highest in *marcha* samples collected from Sikkim (H: 1.74) and lowest in *hamei* samples of Manipur (H: 0.69) indicating higher fungal diversity in *marcha* samples of Sikkim as compared to starters of other states. The diversity index, which considers both the number of species as well as relative abundance of each species for evaluating diversity (Lucas et al., 2017), showed the highest value for *marcha* of Sikkim. Species richness is the number of different species represented in an ecological community, where it reflects the abundances of species or their distributions (Unterseher et al., 2008). Species Richness (R) values in samples of *marcha* and *khekhrii* were recorded as the highest showing more diversity in species level of filamentous molds. Species evenness refers to how equal the community is numerically, ranging from 0 to 1 (Savary et al., 2018) signifying that the value 1.0 in *thiat, hamei,* and *chowan* have a complete evenness in comparison to other starters. Hence diversity index of filamentous fungal community present in dry starters of North East India showed high diversity within the community. It was observed that there was variation in fungal species distribution in each type of amylolytic starters in North East India which determines the quality of the acholic product, preferred by the local consumers. This might be due to varied geographical regions, environmental conditions, and different plant species used in the preparation methods of amylolytic starters. It therefore shows that fungal diversity, present in amylase and alcohol-producing starters, traditionally prepared by ethnic Indian people using their indigenous knowledge of "back-slopping," are morphologically, ecologically, and phylogenetically diverse. Our findings on fungal diversity in amylolytic starters from North East India may supplement the microbial diversity in eco-systems of North East India, which is one of the biodiversity hot spots of the world.

CONCLUSION

Traditionally prepared amylolytic starters are consortia of filamentous fungi, yeasts, and bacteria which are traditionally sub-cultured and preserved using traditional methods of

"back-slopping" by the ethnic people of North East India for production of alcoholic beverages. Yeasts and bacteria present in these starters have already been reported in earlier studies. However, no information on fungal communities and their diversity in Indian amylolytic starters is available. We therefore identified the filamentous molds isolated from *marcha, thiat, humao*, *hamei*, *chowan*, *phut*, *dawdim,* and *khekhrii* based on morphological and sequence-based identifications. We identified seven genera with 16 species represented by *Aspergillus flavus, Aspergillus niger*, *Aspergillus sydowii*, *Aspergillus versicolor*, *Bjerkandera adusta*, *Cladosporium parahalotolerans*, *Mucor circinelloides*, *Mucor indicus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium oxalicum*, *Penicillium polonicum, Rhizopus delemar, Rhizopus microsporus, Rhizopus oryzae,* and *Trametes hirsuta*. Fungal species present in these traditionally prepared dry starters are morphologically, ecologically, and phylogenetically diverse and showed high diversity within the community.

DATA AVAILABILITY STATEMENT

The sequences of the internal transcribed spacers (ITS) region obtained in this study were deposited at the GenBank-NCBI database 6S rRNA sequencing were deposited at GenBank-NCBI numbers: MK396469-MK396484, MK396486-MK396500, MK778442-MK778449, MK796041-MK796045.

AUTHOR CONTRIBUTIONS

AA performed the experiments. JT supervised the experiments and finalized the manuscript.

FUNDING

We are grateful to Department of Biotechnology (DBT), Government of India for financial support. AA is grateful to DBT for the award of the Studentship in DBT-funded Bioinformatics Centre of Sikkim University sanctioned to JT.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.00905/full#supplementary-material

REFERENCES

Abubakar, A., Suberu, H. A., Bello, I. M., Abdulkadir, R., Daudu, O. A., and Lateef, A. A. (2013). Effect of pH on mycelial growth and sporulation of *Aspergillus parasiticus*. *J. Plant Sci.* 1, 64–67. doi: 10.11648/j.jps.20130104.13

Achaya, K. T. (1991). Alcoholic fermentation and its products in ancient India. *Indian J. History Sci.* 26, 123–129.

Adebo, O. A., Kayitesi, E., and Njobeh, P. B. (2019). Reduction of mycotoxins during fermentation of whole grain sorghum to whole grain ting (a Southern African Food). *Toxins* 11:180. doi: 10.3390/toxins11030180

Adekoya, I., Obadina, A., Phoku, J., Nwinyi, O., and Njobeh, P. (2017). Contamination of fermented foods in Nigeria with fungi. *LWT-Food Sci. Technol.* 86, 76–84.

Alsohaili, S. A., and Bani-Hasan, B. A. (2018). Morphological and molecular identification of fungi isolated from different environmental sources in the Northern Eastern desert of Jordan. *Jordan J. Biol. Sci.* 11, 329–337.

Andersen, B., Frisvad, J. C., Søndergaard, I., Rasmussen, I. S., and Larsen, L. S. (2011). Associations between fungal species and water-damaged building materials. *Appl. Environ. Microbiol.* 77, 4180–4188. doi: 10.1128/AEM.02513-10

Anupma, A., Pradhan, P., Sha, S. P., and Tamang, J. P. (2018). Traditional skill of ethnic people of the Eastern Himalayas and North East India in preserving microbiota as dry amylolytic starters. *Indian J. Trad. Know.* 17, 184–190.

Bajaj, I., Veiga, T., van Dissel, D., Pronk, J. T., and Daran, J. M. (2014). Functional characterization of a *Penicillium chrysogenum* mutanase gene induced upon co-cultivation with *Bacillus subtilis*. *BMC Microbiol.* 14:114. doi: 10.1186/1471-2180-14-114

Bavaro, S. L., Susca, A., Frisvad, J. C., Tufariello, M., Chytiri, A., Perrone, G., et al. (2017). Isolation, characterization, and selection of molds associated to fermented black table olives. *Front. Microbiol.* 8:1356. doi: 10.3389/fmicb.2017.01356

Bensch, K., Braun, U., Groenewald, A., and Crous, P. W. (2012). The genus *Cladosporium*. *Stud. Mycol.* 72, 1–401. doi: 10.3114/sim0003

Bora, S. S., Keot, J., Das, S., Sarma, K., and Barooah, M. (2016). Metagenomics analysis of microbial communities associated with a traditional rice wine starter culture (Xaj-pitha) of Assam. India. *3 Biotech.* 6:153. doi: 10.1007/s13205-016-0471-1

Carroll, E., Trinh, T. N., Son, H., Lee, Y. W., and Seo, J. A. (2017). Comprehensive analysis of fungal diversity and enzyme activity in nuruk, a Korean fermenting starter, for acquiring useful fungi. *J. Microbiol.* 55:357. doi: 10.1007/s12275-017-7114-z

Chen, B., Wu, Q., and Xu, Y. (2014). Filamentous fungal diversity and community structure associated with the solid-state fermentation of Chinese Maotai-flavor liquor. *Int. J. Food Microbiol.* 179, 80–84. doi: 10.1016/j.ijfoodmicro.2014.03.011

Chuzho, K., Dkhar, M. S., and Lyngdoh, A. (2017). Wood-rotting fungi in two forest stands of Kohima, North-East India: a preliminary report. *Cur. Res. Environ. Appl. Mycol.* 7, 1–7.

Cilerdzic, J., Stajic, M., Vukojevic, J., Duletic-Lausevic, S., and Knezevic, A. (2011). Potential of Trametes hirsuta to produce ligninolytic enzymes during degradation of agricultural residues. *Bioresearch* 6, 2885–2895.

Daroonpunt, R., Tanasupawat, S., and Keeratipibul, S. (2016). Characterization and amylolytic activity of yeast and mold strains from Thai sweet rice. *Malaysian J Microbiol.* 12, 121–131.

Das, A. J., Miyaji, T., and Deka, S. C. (2017). Amylolytic fungi in starter cakes for rice beer production. *J. Gen. Appl. Microbiol.* 63, 236–245. doi: 10.2323/jgam.2016.11.004

Doi, S. A., Pinto, A. B., Canali, M. C., Polezel, D. R., Chinellato, R. A. M., de Oliveira, A. J. F. C. (2018). Density and diversity of filamentous fungi in the water and sediment of Araçá bay in São Sebastião, São Paulo, Brazil. *Biota Neotrop. Campinas* 18:e20170416. doi: 10.1590/1676-0611-bn-2017-0416

Dung, N. T. P., Rombouts, F. M., and Nout, M. J. R. (2007). Characteristics of some traditional Vietnamese starch-based rice wine fermentation starters (men). *LWT-Food Sci. Technol.* 40, 130–135.

Fernandes, E. G., Pereira, O. L., da Silva, C. C., Bento, C. B. P., and de Queiroz, M. V. (2015). Diversity of endophytic fungi in Glycine max. *Microbiol. Res.* 181, 84–92. doi: 10.1016/j.micres.2015.05.010

Fronteras, J. P., and Bullo, L. L. R. (2017). Raw starch-digesting amylase from *Saccharomycopsis fibuligera* 2074 isolated from bubod starter. *Philippine J. Sci.* 146, 27–35.

Gaddeyya, G., Niharika, P. S., Bharathi, P., and Kumar, P. R. (2012). Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. *Adv. Appl. Sci.* 3, 2020–2026.

Ghosh, B., and Ray, R. R. (2011). Current commercial perspective of Rhizopus oryzae: a review. *J. Appl. Sci.* 11, 2470–2486.

Ghosh, K., Ray, M., Adak, A., Dey, P., Halder, S. K., Das, A., et al. (2015). Microbial, saccharifying and antioxidant properties of an Indian rice based fermented beverage. *Food Chem.* 168, 196–202. doi: 10.1016/j.foodchem.2014.07.042

Gryganskyi, A. P., Golan, J., Dolatabadi, S., Mondo, S., Robb, S., Idnurm, A., et al. (2018). Phylogenetic and phylogenomic definition of Rhizopus species. *G3* 8, 2007–2018. doi: 10.1534/g3.118.200235

Hameed, A., Hussain, S. A., Yang, J., Ijaz, M. U., Liu, Q., Suleria, H. A. R., et al. (2017). Antioxidants potential of the filamentous fungi (*Mucor circinelloides*). *Nutrients* 9:1101. doi: 10.3390/nu9101101

Hammer, Ø, Harper, D. A. T., and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron* 4:9.

Hartanti, A. T., Rahayu, G., and Hidayat, I. (2015). Rhizopus species from fresh tempeh collected from several regions in Indonesia. *HAYATI J. Bios.* 22, 136–142.

Hesseltine, C. W., and Kurtzman, C. P. (1990). Yeasts in amylolytic food starters. *Anal. Inst. Biol. Univ. Nac. Autón. México. Ser. Bot.* 1–7. doi: 10.1016/B978-0-444-52149-1.00187-7

Hesseltine, C. W., and Ray, M. L. (1988). Lactic acid bacteria in murcha and ragi. *J. Appl. Microbiol.* 64, 395–401.

Hesseltine, C. W., Rogers, R., and Winarno, F. G. (1988). Microbiological studies on amylolytic Oriental fermentation starters. *Mycopathology* 101, 141–155.

Horisawa, S., Inoue, A., and Yamanaka, Y. (2019). Direct ethanol production from lignocellulosic materials by mixed culture of wood rot fungi *Schizophyllum commune*. *Bjerkandera adusta*, and *Fomitopsis palustris*. *Ferment* 5:21. doi: 10.3390/fermentation5010021

Huang, Y., Busk, P. K., Grell, M. N., Zhao, H., and Lange, L. (2014). Identification of a β-glucosidase from the Mucor circinelloides genome by peptide pattern recognition. *Enzyme Micro. Technol.* 67, 47–52. doi: 10.1016/j.enzmictec.2014.09.002

Jeyaram, K., Singh, W., Capece, A., and Romano, P. (2008). Molecular identification of yeast species associated with "Hamei" — A traditional starter used for rice wine production in Manipur. India. *Int. J. Food Microbiol.* 124, 115–125. doi: 10.1016/j.ijfoodmicro.2008.02.029

Jeyaram, K., Tamang, J. P., Capece, A., and Romano, P. (2011). Geographical markers for Saccharomyces cerevisiae strains with similar technological origins domesticated for rice-based ethnic fermented beverages production in North East India. *Antonie. Van Leeuwen.* 100, 569–578. doi: 10.1007/s10482-011-9612-z

Ji, Z., Jin, J., Yu, G., Mou, R., Mao, J., Liu, S., et al. (2018). Characteristic of filamentous fungal diversity and dynamics associated with wheat Qu and the traditional fermentation of Chinese rice wine. *Int. J. Food Sci. Technol.* 53, 1611–1621.

Jung, M. J., Nam, Y. D., Roh, S. W., and Bae, J. W. (2012). Unexpected convergence of fungal and bacterial communities during fermentation of traditional Korean alcoholic beverages inoculated with various natural starters. *Food Microbiol.* 30, 112–123. doi: 10.1016/j.fm.2011.09.008

Jurjevic, Z., Peterson, S. W., and Horn, B. W. (2012). *Aspergillus* section Versicolores: nine new species and multilocus DNA sequence based phylogeny. *IMA Fungus* 3, 59–79. doi: 10.5598/imafungus.2012.03.01.07

Karimi, K., and Zamani, A. (2013). Mucor indicus: biology and industrial application perspectives: a review. *Biotechnol. Adv.* 31, 466–481. doi: 10.1016/j.biotechadv.2013.01.009

Karlovsky, P., Suman, M., Berthiller, F., De, Meester J, Eisenbrand, G., Perrin, I., et al. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Res.* 32, 179–205. doi: 10.1007/s12550-016-0257-7

Kumbhare, S. V., Dhotre, D. P., Dhar, S. K., Jani, K., Apte, D. A., Shouche, Y. S., et al. (2015). Insights into diversity and imputed metabolic potential of bacterial communities in the continental shelf of Agatti Island. *PLoS One* 10:e129864. doi: 10.1371/journal.pone.0129864

Lai, X., Zhang, H., Liu, R., and Liu, C. (2015). Potential for aflatoxin B1 and B2 production by *Aspergillus flavus* strains isolated from rice samples. *Saudi J. Biol. Sci.* 22, 176–180. doi: 10.1016/j.sjbs.2014.09.013

Lee, C. H., and Lee, S. S. (2002). Cereal fermentation by fungi. *Appl. Mycol. Biotechnol.* 2, 151–170.

Lee, S. C., Billmyre, R. B., Li, A., Carson, S., Sykes, S. M., Huh, E. Y., et al. (2014). Analysis of a food-borne fungal pathogen outbreak: virulence and genome of a *Mucor circinelloides* isolate from yogurt. *mBio* 5:e01390-14. doi: 10.1128/mBio.01390-14

Li, Y., Zheng, X., Zhang, X., Bao, L., Zhu, Y., Qu, Y., et al. (2016). The different roles of *Penicillium oxalicum* LaeA in the production of extracellular cellulase and β-xylosidase. *Front. Microbiol.* 7:2091. doi: 10.3389/fmicb.2016.02091

Londoño-Hernández, L., Ramírez-Toro, C., Ruiz, H. A., Ascacio-Valdés, J. A., Aguilar-Gonzalez, M. A., Rodríguez-Herrera, R., et al. (2017). Rhizopus oryzae – ancient microbial resource with importance in modern food industry. *Int. J. Food Microbiol.* 257, 110–127. doi: 10.1016/j.ijfoodmicro.2017.06.012

Lucas, R., Groeneveld, J., Harms, H., Johst, K., Frank, K., and Kleinsteuber, S. (2017). A critical evaluation of ecological indices for the comparative analysis of microbial communities based on molecular datasets. *FEMS Microbiol. Ecol.* 93:1. doi: 10.1093/femsec/fiw209

Lutzoni, F., Kauff, F., Cox, C. J., McLaughlin, D., Celio, G., Dentinger, B., et al. (2004). Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American J. Bot.* 91, 1446–1480. doi: 10.3732/ajb.91.10.1446

Lv, X. C., Huang, Z. Q., Zhang, W., Rao, P. F., and Ni, L. (2012a). Identification and characterization of filamentous fungi isolated from fermentation starters for Hong Qu glutinous rice wine brewing. *J. Gen. Appl. Microbiol.* 58, 33–42. doi: 10.2323/jgam.58.33

Lv, X. C., Weng, X., Zhang, W., Rao, P. F., and Ni, L. (2012b). Microbial diversity of traditional fermentation starters for Hong Qu glutinous rice wine as determined by PCR-mediated DGGE. *Food Control* 28, 426–434.

Ly, S., Mith, H., Tarayre, C., Taminiau, B., Daube, G., Fauconnier, M. L., et al. (2018). Impact of microbial composition of Cambodian traditional dried starters (Dombea) on flavor compounds of rice wine: combining amplicon sequencing with HP-SPME-GCMS. *Front. Microbiol.* 9:894. doi: 10.3389/fmicb.2018.00894

Ma, R., Sui, L., Zhang, J., Hu, J., and Liu, P. (2019). Polyphasic characterization of yeasts and lactic acid bacteria metabolic contribution in semi-solid fermentation of *Chinese baijiu* (traditional fermented alcoholic drink): towards the design of a tailored starter culture. *Microorganisms* 7:147. doi: 10.3390/microorganisms705014

Martin, K. J., and Rygiewicz, P. T. (2005). Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC Microbiol.* 5:28. doi: 10.1186/1471-2180-5-28

Mudili, V., Siddaih, C. N., Nagesh, M., Garapati, P., Naveen Kumar, K., Murali, H. S., et al. (2014). Mould incidence and mycotoxin contamination in freshly harvested maize kernels originated from India. *J. Sci. Food Agri.* 94, 2674–2683. doi: 10.1002/jsfa.6608

Nakazato, M., Morozumi, S., Saito, K., Fujinuma, K., Nishima, T., and Kasai, N. (1990). Interconversion of aflatoxin B1 and aflatoxicol by several fungi. *Appl. Environ. Microbiol.* 56, 1465–1470.

Nikkuni, S., Karki, T. B., Terao, T., and Suzuki, C. (1996). Microflora of mana, a Nepalese rice koji. *J. Ferment.Bioengin.* 81, 168–170. doi: 10.1016/0922-338X(96)87597-87590

Odoni, D. I., Tamayo-Ramos, J. A., Sloothaak, J., van Heck, R. G. A., Martins Dos Santos, V. A. P., de Graaff, L. H., et al. (2017). Comparative proteomics of Rhizopus delemar ATCC 20344 unravels the role of amino acid catabolism in fumarate accumulation. *Peer J.* 5:e3133. doi: 10.7717/peerj.3133

Panda, T., Pani, P. K., Mishra, N., and Mohanty, R. B. (2010). A comparative account of the diversity and distribution of fungi in tropical forest soils and sand dunes of Orissa. India. *J. Biodiv.* 1, 27–41.

Pinto, F. C. J., Lima, D. B. D., Agustini, B. C., Dallagassa, C. B., Shimabukuro, M. F., Chimelli, M., et al. (2012). Morphological and molecular identification of filamentous fungi isolated from cosmetic powders. *Brazilian Arch. Biol. Technol.* 55, 897–901.

Piontek, M., Łuszczyňska, K., and Lechów, H. (2016). Occurrence of the toxin-producing *Aspergillus versicolor* Tiraboschi in residential buildings. *Int. J. Environ. Res. Public Health* 13, e862. doi: 10.3390/ijerph13090862

Pitt, J. I, and Hocking, A. D. (2009). *Fungi and Food Spoilage*, 3rd Edn. New York, NY: Springer Dordrecht.

Pradhan, P., and Tamang, J. P. (2019). Phenotypic and genotypic identification of bacteria isolated from traditionally prepared dry starters of the Eastern Himalayas. *Front. Microbiol.* 10:2526. doi: 10.3389/fmicb.2019.02526

Priyanka, S. R., Ramana, M. V., Balakrishna, K., Murali, H. S., and Batra, H. V. (2012). A novel non radioactive PCR-DNA probe for the detection of aflatoxin

producing *Aspergillus* species from major food crops grown in India. *Adv. Microbiol.* 2, 577–586. doi: 10.4236/aim.2012.24075

Qiao, W., Tao, J., Luo, Y., Tang, T., Miao, J., and Yang, Q. (2018). Microbial oil production from solid-state fermentation by a newly isolated oleaginous fungus, Mucor circinelloides Q531 from mulberry branches. *R. Soc. Open Sci.* 5:180551. doi: 10.1098/rsos.180551

Quiroz-Castañeda, R. E., Balcázar-López, E., Dantán-González, E., Martinez, A., Folch-Mallol, J., and Martínez Anaya, C. (2009). Characterization of cellulolytic activities of *Bjerkandera adusta* and *Pycnoporus sanguineus* on solid wheat straw medium. *Electr. J. Biotechnol.* 12, 5–6. doi: 10.2225/vol12-issue4-fulltext-3

Raja, H. A., Miller, A. N., Pearce, C. J., and Oberlies, N. H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. *J. Nat. Prod.* 80, 756–770. doi: 10.1021/acs.jnatprod.6b01085

Ramírez-Camejo, L. A., Zuluaga-Montero, A., Lázaro-Escudero, M. A., Hernández-Kendall, V. N., and Bayman, P. (2012). Phylogeography of the cosmopolitan fungus *Aspergillus flavus*: is everything everywhere?". *Fungal Biol.* 116, 452–463. doi: 10.1016/j.funbio.2012.01.006

Romanelli, A. M., Sutton, D. A., Thompson, E. H., Rinaldi, M. G., and Wickes, B. L. (2010). Sequence-based identification of filamentous basidiomycetous fungi from clinical specimens: a cautionary note. *J. Clin. Microbiol.* 48, 741–752. doi: 10.1128/JCM.01948-09

Rosales, E., Couto, S. R., and Sanromán, M. A. (2005). Reutilisation of food processing wastes for production of relevant metabolites: application to laccase production by *Trametes hirsuta*. *J. Food Eng.* 66, 419–423.

Roslan, R., Rehan, M. M., Kamarudin, K. R., Noor, H. M., Huda-Faujan, N., and Radzi, S. M. (2018). Isolation and identification of amylolytic bacteria from Ragi. *Malaysian Appl. Biol.* 47, 83–88.

Samson, R. A., Hoekstra, E. S., and Frisvad, J. C. (2004). *Introduction to Food and Airborne Fungi*, 7th Edn. Utrecht: Central Bureau Voor Schimmer Cultures (CBS).

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H. W., et al. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Stud. Mycol.* 78, 141–173. doi: 10.1016/j.simyco.2014.07.004

Saori, A., and Keller, N. P. (2011). *Aspergillus flavus*. *Ann. Rev. Phytopathol.* 49, 107–133.

Sarkar, A., Mukherjee, A., Bera, M. K., Das, A., Juyal, N., Morthekai, R., et al. (2016). Oxygen isotope in archaeological bioapatite from India: implications to climate change and decline of Bronze Age Harappan civilization. *Sci. Rep.* 6:26555. doi: 10.1038/srep26555

Savary, R., Villard, L., and Sanders, I. R. (2018). Within-species phylogenetic relatedness of a common mycorrhizal fungus affects evenness in plant communities through effects on dominant species. *PLoS One* 13:e0198537. doi: 10.1371/journal.pone.0198537

Schmitz, A., and Riesner, D. (2006). Purification of nucleic acids by selective precipitation with polyethylene glycol 6000. *Anal. Biochem.* 354, 311–313. doi: 10.1016/j.ab.2006.03.014

Sha, S. P., Jani, K., Sharma, A., Anupma, A., Pradhan, P., Shouche, Y., et al. (2017). Analysis of bacterial and fungal communities in Marcha and Thiat, traditionally prepared amylolytic starters of India. *Sci. Rep.* 7:10967. doi: 10.1038/s41598-017-11609-y

Sha, S. P., Suryavanshi, M. S., and Tamang, J. P. (2019). Mycobiome diversity in traditionally prepared starters for alcoholic beverages in India by high-throughput sequencing method. *Front. Microbiol.* 10:348. doi: 10.3389/fmicb.2019.003482237

Sha, S. P., Suryavanshi, M. V., Jani, K., Sharma, A., Shouche, Y. S., and Tamang, J. P. (2018). Diversity of yeasts and molds by culture-dependent and culture-independent methods for mycobiome surveillance of traditionally prepared dried starters for the production. *Front. Microbiol.* 9:2237. doi: 10.3389/fmicb.2018.02237

Shakeel, Q., Lyu, A., Zhang, J., Wu, M., Li, G., Hsiang, T., et al. (2018). Biocontrol of *Aspergillus flavus* on peanut kernels using Streptomyces yanglinensis 3-10. *Front. Microbiol.* 9:1049. doi: 10.3389/fmicb.2018.01049

Sharifyazd, S., and Karimi, K. (2017). Effects of fermentation conditions on valuable products of ethanolic fungus *Mucor indicus*. *Electr. J. Biotechnol.* 30, 77–82.

Shoubao, Y., Xiangsong, C., and Jiaquan, G. (2019). Bacterial and fungal diversity in the traditional Chinese strong flavour liquor Daqu. *J. Inst. Brew.* 125, 443–452. doi: 10.1002/jib.574

Shrivastava, K., Greeshma, A. G., and Srivastava, B. (2012). Biotechnology in tradition – a process technology of alcoholic beverages practiced by different tribes of Arunachal Pradesh. North East India. *Indian J. Trad. Knowl.* 11, 81–89.

Singh, N. L., Ramprasad Mishra, P. K., Shukla, S. K., Kumar, J., and Singh, R. (2010). Alcoholic fermentation techniques in early Indian tradition. *Indian J. History Sci.* 45, 163–173.

Suesse, A. R., Norton, G. A., and van Leeuwen, J. (2016). Pilot-scale continuous-flow hydrothermal liquefaction of filamentous fungi. *Energy Fuels* 30, 7379–7386. doi: 10.1021/acs.energyfuels.6b01229

Tamang, J. P. (2010). *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values*. New York, NY: CRC Press.

Tamang, J. P., Cotter, P., Endo, A., Han, N. S., Kort, R., Liu, S. Q., et al. (2020). Fermented foods in a global age: east meets west. *Comprehen. Rev. Food Sci. Food Saf.* 19, 184–217. doi: 10.1111/1541-4337.12520

Tamang, J. P., Dewan, S., Tamang, B., Rai, A., Schillinger, U., and Holzapfel, W. H. (2007). Lactic acid bacteria in hamei and marcha of North East India. *Indian J. Microbiol.* 47, 119–125. doi: 10.1007/s12088-007-0024-8

Tamang, J. P., and Sarkar, P. K. (1995). Microflora of murcha: an amylolytic fermentation starter. *Microbios.* 81, 115–122.

Tamang, J. P., Sarkar, P. K., and Hesseltine, C. W. (1988). Traditional fermented foods and beverages of Darjeeling and Sikkim - a review. *J. Sci. Food Agri.* 44, 375–385.

Tamang, J. P., and Thapa, S. (2006). Fermentation dynamics during production of bhaati jaanr, a traditional fermented rice beverage of the Eastern Himalayas. *Food Biotechnol.* 20, 251–261.

Tamang, J. P., Watanabe, K., and Holzapfel, W. H. (2016). Diversity of microorganisms in global fermented foods and beverages. *Front. Microbiol.* 7:377. doi: 10.3389/fmicb.2016.00377

Thakur, N., Saris, P. E., and Bhalla, T. C. (2015). Microorganisms associated with amylolytic starters and traditional fermented alcoholic beverages of North Western Himalayas in India. *Food Biosci.* 11, 92–96.

Thanh, V. N., Mai, L. T., and Tuan, D. A. (2008). Microbial diversity of traditional Vietnamese alcohol fermentation starters (banh men) as determined by PCR-mediated DGGE. *Int. J. Food Microbiol.* 128, 268–273. doi: 10.1016/j.ijfoodmicro.2008.08.020

Thapa, S., and Tamang, J. P. (2004). Product characterization of kodo ko jaanr: fermented finger millet beverage of the Himalayas. *Food Microbiol.* 21, 617–622.

Thapa, S., and Tamang, J. P. (2006). Microbiological and physio-chemical changes during fermentation of kodo ko jaanr, a traditional alcoholic beverage of the Darjeeling hills and Sikkim. *Indian J. Microbiol.* 46, 333–341.

Tsang, C. C., Tang, J. Y., Lau, S. K., and Woo, P. C. (2018). Taxonomy and evolution of *Aspergillus*, *Penicillium* and *Talaromyces* in the omics era–Past, present and future. *Computer Struct. Biotech.* 16, 197–210. doi: 10.1016/j.csbj.2018.05.003

Tsuyoshi, N., Fudou, R., Yamanaka, S., Kozaki, M., Tamang, N., Thapa, S., et al. (2005). Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation. *Int. J. Food Microbiol.* 99, 135–146. doi: 10.1016/j.ijfoodmicro.2004.08.011

Umesha, S., Manukumar, H. M., and Raghava, S. (2016). A rapid method for isolation of genomic DNA from food-borne fungal pathogens. 6, 123. doi: 10.1007/s13205-016-0436-4

Unterseher, M., Schnittler, M., Dormann, C., and Sickert, A. (2008). Application of species richness estimators for the assessment of fungal diversity. *FEMS Microbiol. Lett.* 282, 205–213. doi: 10.1111/j.1574-6968.2008.01128.x

Wagner, L., Stielow, J. B., de Hoog, S., Bensch, K., Schwartze, V., Voigt, K., et al. (2019). A new species concept for the clinically relevant *Mucor circinelloides* complex. *Personia* 44, 67–97.

Wang, J., Chio, C., Chen, X., Su, E., Cao, F., Jin, Y., et al. (2019). Efficient saccharification of agave biomass using *Aspergillus niger* produced low-cost enzyme cocktail with hyperactive pectinase activity. *Biores. Technol.* 272, 26–33. doi: 10.1016/j.biortech.2018.09.069

Wei, H., Wang, W., Yarbrough, J. M., Baker, J. O., Laurens, L., and Van Wychen, S. (2013). Genomic, proteomic, and biochemical analyses of oleaginous *Mucor circinelloides*: evaluating its capability in utilizing cellulolytic substrates for lipid production. *PLoS One* 8:e71068. doi: 10.1371/journal.pone.0071068

Xing, J. H., Sun, Y. F., Han, Y. L., Cui, B. K., and Dai, Y. C. (2018). Morphological and molecular identification of two new Ganoderma species on *Casuarina*

equisetifolia from China. *Mycol. Keys* 34, 93–108. doi: 10.3897/mycokeys.34.22593

Xu, J. (2016). Fungal DNA barcoding. *Genome* 59, 913–932.

Yang, S., Lee, J., Kwak, J., Kim, K., Seo, M., and Lee, Y. W. (2011). Fungi associated with the traditional starter cultures used for rice wine in Korea. *J. Korean Soc. Appl. Biol. Chem.* 54, 933–943.

Zhang, Y., Fraatz, M. A., Müller, J., Schmitz, H. J., Birk, F., Schrenk, D., et al. (2015). Aroma characterization and safety assessment of a beverage fermented by Trametes versicolor. *J. Agric. Food Chem.* 63, 6915–6921. doi: 10.1021/acs.jafc.5b02167

Zheng, X. W., Yan, Z., Han, B. Z., Zwietering, M. H., Samson, R. A., Boekhout, T., et al. (2012). Complex microbiota of a Chinese "Fen" liquor fermentation starter (Fen-Daqu), revealed by culture-dependent and culture-independent methods. *Food Microbiol.* 31, 293–300. doi: 10.1016/j.fm.2012.03.008

Zhou, B., Ma, C., Wang, H., and Xia, T. (2018). Biodegradation of caffeine by whole cells of tea-derived fungi *Aspergillus sydowii*, *Aspergillus niger* and optimization for caffeine degradation. *BMC Microbial.* 18:53. doi: 10.1186/s12866-018-1194-8

Zulkifli, N. A., and Zakaria, L. (2017). Morphological and molecular diversity of *Aspergillus* from corn grain used as livestock feed. *HAYATI J. Biosci.* 24, 26–34. doi: 10.1016/j.hjb.2017.05.002

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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