Studies on Yeasts Diversity in Some Amylolytic Starters of North East India Using Culture-Dependent and Culture-Independent Techniques

A Thesis Submitted

To

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In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

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Dedicated to my beloved parents

Shri Ram Prasad Sha

&

Late Smt. Shanti Devi

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"Studies on Yeasts Diversity in Some Amylolytic Starters of North East India Using Culture-Dependent and Culture-Independent Techniques"

Submitted by **Shri Shankar Prasad Sha** under the supervision of **Professor Dr. Jyoti Prakash Tamang,** Professor, Department of Microbiology, Dean School of Life Sciences, Sikkim University, Gangtok, 737102, Sikkim, India.

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Date: 27/06/2018 **Shankar Prasad Sha**

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RESULTS

DISCUSSION

Food fermentation is one of the oldest traditional technologies for production of edible products in the development of human civilization (Hesseltine 1983; Steinkraus 1996; Tamang 2010a). Fermented foods are defined as food products prepared by the people using their indigenous knowledge of food fermentation from locally available raw materials of plant or animal source either naturally or by adding starter culture(s) containing functional microorganisms which modify the substrates biochemically and organoleptically into edible products that are socially and culturally acceptable to the consumers (Tamang 2010b; Tamang et al. 2016a). Fermented beverages and alcoholic drinks are socially and culturally acceptable products for consumption, drinking, entertainment, customary practices and religious purposes (Tamang, 2010c). Drinking of alcoholic beverages and distilled alcohol are widespread interest enhancing the pleasure of eating and have nutritional significance (Darby 1979). Consumption of alcoholic drinks in India has been mentioned in the *Ramayana* during 300-75 BC (Prakash 1961). Ethnic alcoholic beverages drinks have strong ritualistic importance among the ethnic people in Asia and Africa where social activities require provision and consumption of appreciable quantities of alcohol, whereas wine has a deep-rooted cultural history for the European as well as Mediterranean ethnic people (Pretorius 2000; Tamang and Samuel 2010). Although a diverse range of alcoholic product is available, a general scheme for their production can be presented as (i) selection of the raw material (ii) processing of the raw material to give a fermentable extract (iii) alcoholic fermentation by yeast, principally by strains of *Saccharomyces cerevisiae* (iv) distillation of the fermented material to give the distillate product and (v) post-distillation processing (Watson 1993; Bluhm 1995). About ten different categories of global alcoholic beverages have been reported by Tamang (2010c):

1) Non-distilled and unfiltered alcoholic beverages produced by amylolytic starters e.g., *kodokojaanr* (fermented finger millets) (Thapa and Tamang 2004) and *bhaatijaanr* (fermented rice) of India and Nepal (Tamang and Thapa 2006), *makgeolli* (fermented rice) of Korea (Jung et al. 2012).
2) Non-distilled and filtered alcoholic beverages produced by amylolytic starters e.g., *saké* of Japan (Kotaka et al. 2008).
3) Distilled alcoholic beverages produced by amylolytic starter e.g., *shochu*of Japan, and *soju* of Korea (Steinkraus 1996).
4) Alcoholic beverages produced by involvement of amylase in human saliva e.g., *chicha* of Peru (Vallejo et al. 2013).
5) Alcoholic beverages produced by mono- (single-strain) fermentation e.g., beer (Kurtzman and Robnet 2003).
6) Alcoholic beverages produced from honey e.g., *tej* of Ethiopia (Bahiru et al. 2006).
7) Alcoholic beverages produced from plant parts e.g., *pulque* of Mexico (Lappe-Oliveras et al. 2008), *toddy* of India (Shamala and Sreekantiah 1988) and *kanji* of India (Kingston et al. 2010).
8) Alcoholic beverages produced by malting (germination) e.g., *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. 2013a).
9) Alcoholic beverages prepared from fruits without distillation e.g., wine, cider.
10) Distilled alcoholic beverages prepared from fruits and cereals e.g., whisky and brandy.

Malting process for alcohol production is rare or unknown in Asia, whereas wine making is not a tradition in Asia (Nout and Aidoo 2002; Tamang 2016) since fruits

are eaten directly without extracting into juice or fermenting into wine (Tamang 2010c). In Asia preparation of amylolytic (related to conversion of starch to sugar) starter is an innovative back-sloping technique of cultivation of native microbiota in the form of dry, flattened, or round balls made up of rice/wheat for production of different traditional alcoholic beverages (Hesseltine 1991;Tamang, 2010c), locally known as *marcha* in India, Nepal and Bhutan, *benh men* in Vietnam, *bubod* in the Philippines, *chiu/chu/daque* in China and Taiwan, *loogpang* in Thailand, *ragi* in Indonesia, and *nuruk* in Korea (Steinkraus 1996; Tamang and Fleet 2006; Tamang 2016).Traditional methods of preparation of Asian amylolytic dry starters are similar with negligible variation in terms of wrapping materials, size and shapes, incubation period of particular starters. Asian amylolytic starter cultures are of three different types based on use of inocula is used: First type: Starch to sugar, Second type: Sugar to alcohol and Third type: Alcohol to organic acid (Hesseltine et al. 1988; Steinkraus 1996; Fleet 1998; Tamang and Fleet 2009).The microflora that are associated with traditionally prepared Asian amylolytic starter cultures include starch-degrading genera of molds *Actinomucor, Amylomyces, Aspergillus, Mucor, Neurospora, Penicillium, Rhizopum* etc.(Hesseltine et al. 1988; Tamang et al. 1988; Nikkuni et al. 1996; Nout and Aidoo 2002; Chen et al. 2014; Tamang et al. 2016a); amylolytic and alcohol-producing yeasts genera mostly *Candida, Debaryomyces, Dekkera, Galactomyces, Geotrichum, Hansenula, Hanseniaspora, Issatchenkia, Kazachstania, Kluyveromyces, Pichia, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Torulaspora, Torulopsis, Wickerhamomyces,* and *Zygosaccharomyces* (Hesseltine and Kurtzman 1990; Tamang and Sarkar 1995; Tsuyoshi et al. 2005; Tamang et al. 2007; Jeyaram et al. 2008; Lv et al. 2012, 2013; Chakrabarty et al. 2014; Sha et al 2017) and few genera of bacteria mostly

Pedococcus, Lactobacillus, etc. (Hesseltine and Ray 1988; Tamang and Sarkar 1995; Tamang et al. 2007; Chakrabarty et al. 2014).

The concept of ethno-microbiology is very important component in the modern food microbiology since this traditional technology involves the process of conservation and crude sub-culturing of essential and functional microbiota or microbiome using back-sloping method by the ethnic people for centuries (Tamang 2010a). Traditionally the ethnic people know how to get the desirable products using their indigenous knowledge for production of foods for consumption. They did not know what was the scientific explanation of fermentation mechanisms and identity of functional microorganisms. Understanding the ethno-microbiology in terms of culture-dependent and independent methods to document a complete profile of microorganisms, and also to study both inter- and intra-species diversity within a particular genus or among genera (Yan et al. 2013). Molecular identification is emerging as an accurate and reliable identification tool for identification of both culture-dependent and culture-independent microorganisms from fermented foods (Dolci et al. 2015). Due to limitation of only isolation of culturable microorganisms, the culture-dependent methods may not detect the whole microbial community in foods. However, the culture-independent methods by extracting whole genomic DNA directly from small amount (<1 g) samples of fermented food may detect the whole microbial communities in food samples (Puerari et al. 2015). Culture-independent methods including pyrosequencing, PCR-denaturing gradient gel electrophoresis (DGGE) analysis, and recently next generation sequencing such as High throughput metagenomic amplicon sequencing may serve to give more insight into microbial ecology of natural food fermentation with increased accuracy, and relatively short

period of time (Ercolini 2004, Alegría et al. 2011; Chen et al. 2014; Puerari et al. 2015; Tamang et al. 2016a; Shangpliang et al. 2018).

Analysis of the Internal Transcribed Spacer (ITS) region has been widely applied in explorations of diversity of yeasts associated with various traditional fermented foods (Caggia et al. 2001; Las Heras-Vazquez et al. 2003), which may provide the fast and easy means for accurate identification at species level (Esteve-Zarzoso et al. 1999), due to higher sequence variation, (Iwen et al. 2002; Korabecna 2007; Susan Slechta et al. 2012). The PCR-DGGE analysis is the most commonly used among the culture-independent fingerprinting technique which is based on the separation of amplicons (PCR-products) of the same size but having different sequences of 16SrRNA and 26SrRNA amplicons (Cocolin et al. 2000; Ercolini 2004). The PCR-DGGE approach is used to investigate the yeast diversity during commercial wine fermentations (Cocolin et al. 2001). Moreover, recently developed bioinformatics tools helps to recover microbial genomes directly from metagenomes, allowing strain-level identification during the process and genomic comparison (Eren et al. 2015; Scholz et al. 2016). Rapid evolution in high-throughput sequencing techniques has enabled researchers to have increased accuracy, high throughput sequencing tool, with reasonably low cost and in relatively short period of time (Cocolin et al. 2013; Mayo et al. 2014).

Some researchers have reported the microbial community in some traditionally prepared starters cultures and traditional alcoholic drinks of some countries by using PCR-DGGE analysis such as Chinese amylolytic starter *yaoqu*/*hongqu* (Lv et al. 2013) and *daqu* (Chen et al. 2014), sorghum-based alcoholic beverage of Benin *tchoukoutou* (Greppi et al. 2013), *chicha*, ethnic alcoholic beverage of Brazil (Puerari et al. 2015). Metagenomic studies using high-throughput

sequencing techniques of various fermented milk products have shown a realistic view of the microbial community structure involved in the natural fermentation (Dobson et al. 2011; Quigley et al. 2012; Liu et al. 2018).

There are eight states located in North East regions of India commonly known as North East (www.northeasttourism.gov.in). All eight states of North East have various varieties of traditionally prepared amylolytic starters prepared by different linguistic ethnic groups of people that include *marcha* of Sikkim, *humao* of Assam, *hamei* of Manipur, *chowan* of Tripura, *thiat* of Meghalaya, *khekhrii* of Nagaland, *dowdim* of Mizoram and *phut* of Arunachal Pradesh. These starter cultures except *khekhrii* of Nagaland are traditionally prepared from soaked rice with some wild herbs, previously prepared starter powder (1-2%) as an inoculum (back-sloping), and then mixtures are ground in a wooden mortal with addition of water to make a thick dough which are kneaded into round to flattened balls/cakes of different size and shape, covered with fern fronds/paddy straws/jute sags, fermented at room temperature for 1-3 days; and fresh balls/cakes are sun dried for few days (Tamang et al. 1996; Tamang 2010a; Anupma et al. 2018). *Khekhrii/khrie* of Nagaland is prepared by naturally fermenting germinated sprouted-rice grains and then sun-dried to use as dry starter culture to prepare *zutho*, local alcoholic beverage.

There are very limited reports on microbial profiles of the above listed amylolytic starters of North East India except *marcha* (Hesseltine et al. 1988; Tamang et al. 1988; Hesseltine and Kurtzman 1990; Tamang and Sarkar 1995; Tsuyoshi et al. 2005; Tamang et al. 2007), *hamei* (Jeyaram 2008, 2011; Tamang et al. 2007) and *humao* (Chakrabarty et al. 2014) Based on the above mentioned research gaps, the present Thesis was designed to accomplish the following approved Objectives.

1) Collection of samples of traditionally prepared amylolytic starters of North East India such as *Marcha, Humao, Ipoh, Hamei, Thiat,* etc for isolation by culture-dependent method and determination of microbial population (cfu/g).
2) Isolation and screening of yeasts from collected samples by culture-independent method using technique of PCR-DGGE to determine the yeast community present in traditionally prepared amylolytic starters.
3) Identification of yeasts by phenotypic and molecular techniques. Results of molecular tools with those obtained by the cultural methods will be corroborated.
4) Determination of amylolytic activities and alcohol producing abilities of identified dominant yeasts.

Preliminary analysis of microbial load in traditionally prepared starters of North East India, fungi mostly yeasts and filamentous molds ($>10^7$cfu/g) are predominance over bacteria Hence, we aimed to investigate the yeast and fungal communities in traditionally prepared amylolytic starters of all eight states of India viz: *marcha* (Sikkim), *thiat* (Meghalaya), *hamei* (Manipur), *phut* (Arunachal Pradesh), *chowan* (Tripura), *dowidim* (Mizoram), *humao* (Assam) and *khekhrii* (Nagaland) by culture-dependent methods such as phenotypic characterizations, Biolog system, and ITS-PCR; and culture-independent methods including PCR-DGGE. We also analyzed microbial community consisting of all fungal, yeast and bacteria using culture-independent technique of High-throughput amplicon sequencing from *marcha* of Sikkim and *thiat* of Meghalaya, respectively. We also studied their enzymatic activities and alcohol productivity. This is the first report on complete profile of yeast and filamentous fungi associated with traditionally prepared ethnic amylolytic starters

of North East India using ITS-PCR, PCR-DGGE and High-throughput sequencing techniques.

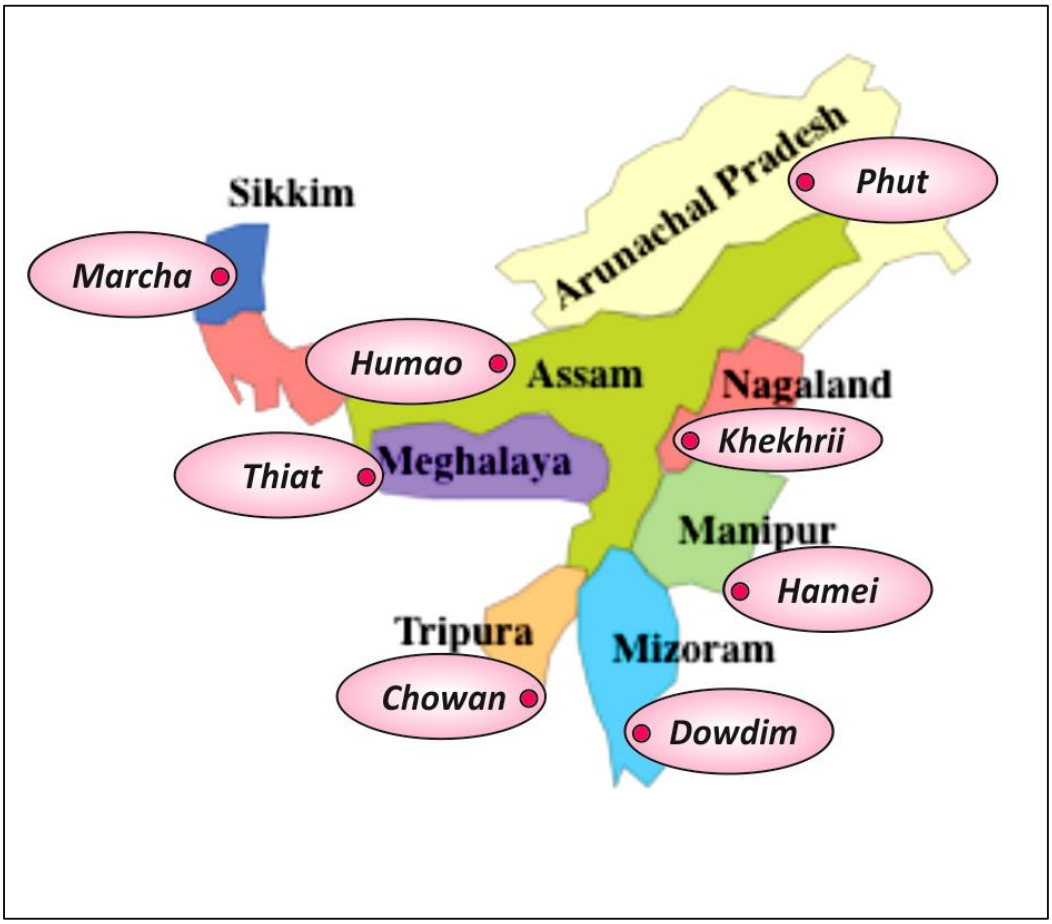


Figure A. Map showing different collection sites of traditionally prepared amylolytic starters of North East India.

In Asia amylolytic starter culture prepared from the growth of filamentous fungi and yeasts on raw or cooked cereals are more commonly used (Haard et al. 1999; Tamang 2016). The use of mixed amylolytic starters might have its origins during the time of Euchok, the daughter of the legendary king of Woo of China, known as the Goddess of rice-wine in Chinese culture in 4000 BC (Lee 1984; Lee and Kim 2016). The first documentation of *chu*, a Chinese amylolytic starter, is very similar to *marcha* of the Himalayas (Tamang 2010a), was reported in Shu-Ching document written during Chou dynasty (1121-256 BC), in which it is reported that *chu* is essential for making alcoholic beverages (Haard et al. 1999). According to the text *Chhi Min Yao Shu*, written by Chia Ssu-Hsieh of Late Wei kingdom between 533 and 544 AD, many methods of preparation of *chu* were described (Yoon 1993; Huang 2000). The use of *chu*, a Chinese amylolytic starter for rice-based alcoholic beverage production was commonly practiced in the Spring and Fall and Warrior Periods of China during 6th to 7th centuries B.C. and the beginning of the Three Nations' Periods in Korea during 1st century BC to 2nd century AD (Lee 1995). It might have transferred from Korea to Japan in the 3rd century AD according to *Kojiki*, or Chin, whose memorial document is kept in a shrine at Matsuo or Matsunoo, Taisha, Kyoto, Japan (Lee 1995). The process of cereal alcohol fermentation using mold starters was well established in the year of 1000 BC and forty three different types of cereal wines and beers were described with detailed processing procedures in *Chhi Min Yao Shu* (Haard et al. 1999). According to this document *chu* was prepared from barley, rice and wheat (Yoon 1993). Ten different types of *chu* were described in *Chhi Min Yao Shu* (Yoon 1993; Huang 2000), all of which were used for the fermentation of alcoholic beverages in China. Cake type *ping-chu* is similar to *nuruk* of Korea, and granular type *san-chu* is similar to *koji* of Japanese (Yoon 1993). According to Yokotsuka

(1985), *chu* Chinese starter may either be white probably due to *Rhizopus* and *Mucor or* yellow (*huang*) possibly due to *Aspergillus oryzae. Nu-chu* is prepared by using cooked rice, which is further shaped into a cake and then cultured with molds (Yokotsuka 1985). Wheat *chu* starter originated from the Northern of China and the Korean Peninsular, while rice *chu* starter originated in the South China (Haard et al. 1999). The word *ragi* of Indonesian was first time noted on an ancient inscription called the Kembang Arum, near Yogyakarta in Java of Indonesia around 903 AD (Astuli 1999). In Asia production technique of ethnic starter cultures to make alcoholic beverages is usually kept secret and the indigenous knowledge of processing is not easily passed on. However, the protected hereditary right of making ethnic mixed starters is passed to daughter by mothers, and she carries the indigenous knowledge to in-laws after marriage. Traditionally preparation of ethnic mixed starters is done exclusively by women, *marcha* is prepared by the Limboo and Rai castes of the Nepali, *ragi* by Indonesian, *loogpang* by ethnic Thai, *nuruk* by ethnic Koreas, and *bubod* by the Filipino (Tamang 2010a). Asian ethnic people traditionally prepare three major types of mixed amylolytic starters to convert cereal starch to sugars and subsequently to alcohol and organic acids are practiced in Asia (Steinkraus 1983; Hesseltine et al. 1988; Fleet 1998; Tamang and Fleet 2009).

Type I: Traditional practice of sub-culturing by back-sloping for preservation of essential native microbiota consisting of consortia of yeasts, molds and bacteria, in the form of dry, flattened, or round balls amylolytic starters (related to conversion of starch to sugar), 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 (Tamang 2010a). Consortia of mycelia or filamentous molds, amylolytic and alcohol-producing yeasts and lactic acid bacteria

(LAB) with rice or wheat as the base in the form of dry, round to flattened balls of various sizes. The starter is inoculated with previous starter. This mixed flora is allowed to develop for a short time, then dried, and used to make either alcohol or fermented foods from starchy materials. 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; Dung et al. 2007), 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. 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.

Type II: A combination of *Aspergillus oryzae* and *A. sojae* are used in the form of starter called *koji* in Japan to produce alcoholic beverages including *saké. Koji* also produces amylases that convert starch to fermentable sugars, which are then used for the second stage yeast fermentation to make non-alcoholic fermented soybean product called *miso* and *shoyu*, while proteases are formed to break down the soybean protein.

Type III: Whole-wheat flour with its associated flora is moistened and made into large compact cakes, which are incubated to select certain desirable microorganisms. The cakes are used to inoculate large masses of starchy material, which is then fermented to produce alcohol. This type of starter contains yeasts and filamentous molds, and is mostly used in China for alcohol production. A list of

common traditionally prepared amylolytic starters and their alcoholic products of Asia is shown in Table A.

Table A: Amylolytic starters of Asia and their alcoholic products

Starter Culture	Substrate	Nature and use	Area	References
Amou/*pe rokkushi*	Rice and wild herbs	To ferment rice into alcoholic beverage- *jou*	Bodoland, Assam, India	Das et al. (2017)
Bakhar	Rice flour, ginger	To ferment rice into alcoholic beverage- *pachwai*	India (Himachal Pradesh)	Hutchinson and Ram Ayyar (1925)
Balam	Roasted wheat flour and spices	To ferment alcoholic beverage- *jaan*	India (Uttarakhand)	Roy et al. (2004)
Banh men	Rice, wild herbs, spices	To ferment ricemaize/cassava into alcoholic beverage- *ruou nep chan*	Vietnam	Dung et al. (2007)
Bubod	Rice, wild herbs	To ferment sugar cane into alcoholic beverage- *basi*	The Philippines	Hesseltine and Kurztman (1990)
Chiu/chu, *yao qu* and *hong qu*	Rice, wild herbs	To ferment rice into alcoholic beverage- *Hong qu*. Glutinous rice wine, *Shaoxing* rice wine and *Shandong Jimo* millet wine.	China and Taiwan	Lv et al. (2013)
Dhehli	Herbal mixture of 36 herbs and roasted barley flour	Starter to ferment alcoholic beverage- *sura*	India (Himachal Pradesh)	Thakur et al. (2004)
daqu	Glutinous rice, wild herbs	Starter to ferment alcoholic beverage- *fen*	China	(Chen et al. 2014).
Hamei	Rice, wild herbs	To ferment rice into alcoholic beverages- *atingba*	India (Manipur)	Jeyaram et al. (2009) and Singh and Singh, 2006.
Humao	Rice, barks of wild plants	Dry, flat, cake-like starter for *judima* production	India (Assam)	Chakrabarty et al. (2014)
Ipoh/Siye	Rice and powder of seeds and bark	Starter to ferment alcoholic beverages - *apong* and *ennog*	India (Arunachal Pradesh)	Tiwari and Mahanta (2007)

	of locally available plants			
Keem	Wheat; plants	Starter to ferment alcoholic beverages - *soor*	India (Himachal Pradesh)	Rana et al. (2004)
Khekhrii	Germinated rice	Starter to ferment alcoholic beverages - *zutho/zhuchu*	India (Nagaland)	Jamir and Rao (1990), Jamir and Deb (2014)
loogpang	Rice, wild herbs	*Khao-maak, krachae, nam khao, ou, sato*	Thailand	Vachanavinich et al. 1994)
Maae/dombae/buh/puh	Rice, Spices, herbs	To ferment rice into alcoholic beverage- *sombai*.	Cambodia	Yamamoto and Matsumoto (2011)
Medombae	Rice, Spices, herbs	To ferment rice into alcoholic beverage- *sombai*.	Cambodia	Chay et al. (2017) and Chim et al. (2015)
Malera/treh	Wheat flour	Starter to ferment *bhatooru/chilra*	India (Himachal Pradesh)	Savitri and Bhalla (2007)
Mod pitha	Rice and 31 plant materials	Starter to ferment alcoholic beverages - *sujen*	India (Assam and Arunachal Pradesh)	Deori et al. (2007)
Marcha	Rice, wild herbs, spices	Dry, mixed starter to ferment alcoholic beverages	India (Darjeeling hills, Sikkim, North East)	Tamang and Sarkar (1995)
Nuruk	Rice, herbs	*Takju, sojo, yakju*	Korea	Jung et al. (2012)
Pham/phab	Rice and leaves of *Solanum khasianum*	Starter to ferment alcoholic beverages - *themsing, chhang, arrak, kinnauri*	India (Arunachal Pradesh, Jammu and Kashmir, Himachal Pradesh)	Singh et al. (2007), Angmo and Bhalla (2014)
Ragi	Rice, herbs	To ferment cassava/rice into mild-alcoholic and sweet beverage- *tapé-kekan, brem*	Indonesia	Surono (2016)
Ranu dabai	Rice, herbs	Starter to ferment alcoholic beverages- *jhara* or *haria*	India (West Bengal)	Ghosh and Das (2004)
Ranu goti	Rice, herbs	Starter to ferment alcoholic beverages - *handia* and *mahua*	India (Central India)	Kumar and Rao (2007)

Thiat	Rice powder, powder of *Amomum aromaticum* Roxb. leaves	Starter to ferment alcoholic beverage - *kiad*	India (Meghalaya)	Samati and Begum (2007)
Vekur pitha	Rice, leaves of some local plants	Starter to ferment alcoholic beverages - *ahom*	India (Assam)	Saikia et al. (2007)
Xaj-pitha	Rice, leaves of some local plants	Starter to ferment alcoholic beverages - *xaj*	India (Assam)	Bora et al. (2016)

AMYLOLYTIC STARTERS

Amou/*perokkushi*

Amou/*perokkushi* is amylolytic starters of Assam for preparation of rice-based alcoholic beverage in Assam, by the Deori and Bodo communities, respectively (Das et al. 2017). They identified the amylolytic fungi, based on the sequencing of their internal transcribed spacer (ITS) regions, as *Amylomyces rouxii* and *Rhizopus oryzae*, and both the strains showed the ability to breakdown and saccharify starch (Polysaccharides). The glucoamylase activity was considerably high in *A. rouxii* (14.92 mmol/min) as compared to *R. oryzae* (1.41 mmol/min), whereas a-amylase activity was observed to be closely related, i.e. 7.02 and 6.09 unit/mL, respectively. They used SDS-PAGE to determine molecular size of the glucoamylase enzymes revealed the production of two distinct units of 59 kDa and 31 kDa by *A. rouxii*, and one unit of 72 kDa by *R. oryzae* (Das et al. 2017).

Bakhar

Bakhar is a starter culture used to make *pachwai*, rice wine in eastern part of India and contains *Rhizopus* sp., *Mucor* sp., and at least one species of yeast (Hutchinson and Ram-Ayyar 1925). Ginger and other plant materials are dried, ground and added to rice flour. Water is added to make a thick paste and a small round cake of 1.0-1.5 cm in diameter are formed and inoculated with powdered

cakes from previous batches. The cakes are then wrapped in leaves, allowed to ferment for 3 days and then sun-dried (Hutchinson and Ram Ayyar 1925). Ray (1906) reported the presence of *Saccharomyces cerevisiae* in *bakhar*.

Balam

Balam is traditionally prepared wheat based amylolytic starter of Uttaranchal used for preparation of *jann,* during the preparation of *balam* first the raw wheat is washed and sun dried, then this is ground into flour and then it is roasted over fire and removed before it becomes turns brown in color. The roasted wheat flour is then mixed properly with various plants spices like *Cinnamomum zeylanicum, elachi* (*Amomum subulatum*), *Piper longum* (*kalimirch*), seeds of *Ficus religiosa* (*papal*) and leaves of wild chilies (*mirchi-ghash*). In this mixture, old powder of *balam* is also added. The addition of old *balam* starter powder is a must, without addition of this old starter production of fresh *balam* is not possible. The whole mixture, which is prepared, is now thoroughly mixed with the required amount of water and a thick paste is prepared. This prepared mass is then pressed between palms to make *balam* balls of the different required size. These different sized wet balls are dried in shade and then stored for future use for a long period of time (Roy et al. 2004).

Banh men

Banh men/men is the traditionally prepared amylolytic starers of Vietnam (Dung et al. 2007). The diversity of yeasts (*Candida tropicalis, Clavispora lusitaniae, Pichia anomala, Pichia ranongensis, Saccharomycopsis fibuligera, Sacch. cerevisiae, Issatchenkia* sp.); filamentous molds (*Absidia corymbifera,*

Amylomyces rouxii, Botryobasidium subcoronatum, Rhizopus oryzae, Rhi. microsporus, Xeromyces bisporus); LAB (*Ped. pentosaceus, Lb. plantarum, Lb. brevis, Weissella confusa, Weissella paramesenteroides*); amylase-producing bacilli (*Bacillus subtilis, B. circulans, B. amyloliquefaciens, B. sporothermodurans*); and acetic acid bacteria (*Acetobacter orientalis, A. pasteurianus*) were present in *men*, a starter culture of Vietnam (Dung et al. 2006, 2007; Thanh et al. 2008).The diversity of fungi and bacteria associated with Vietnamese ethnic amylolytic starters, *banh men* was studied by PCR-DGGE. The fungal population of the *banh men* was consistent with little variation among samples. It mainly consisted of amylase producers (*Rhizopus oryzae, R. microsporus, Absidia corymbifera, Amylomyces* sp., *Saccharomycopsis fibuligera*), ethanol producers (*Saccharomyces cerevisiae, Issatchenkia* sp., *Pichia anomala, Candida tropicalis, P. ranongensis, Clavispora lusitaniae*), and opportunistic contaminants (*Xeromyces bisporus, Botryobasidium subcoronatum*). The bacterial population of starters was highly variable in species composition and dominated by lactic acid bacteria (LAB). The most frequent LAB were, *Lactobacillus plantarum, L. brevis, Pediococcus pentosaceus, Weissella confusa* and *W. paramesenteroides*. Species of amylase-producing *Bacillus* (*Bacillus subtilis, B. circulans, B. amyloliquefaciens, B. sporothermodurans*), acetic acid bacteria (*Acetobacter orientalis, A. pasteurianus*) and environment contaminants/plant pathogens (*Burkholderia ubonensis, Ralstonia solanacearum, Pelomonas puraquae*) (Dung et al. 2006; Thanh et al. 2008).

Bubod

Bubod is used as a starter in the Philippines (Tanimura et al. 1977; Elegado 2016). Rice and ginger are powdered, and mixed thoroughly with enough water to have a consistency that permits rolling the material into a ball and flattening it. The discs are coated with 1-3 month old *bubod* and incubated in rice straw for 36 h at room temperature and sun-dried. Tanimura et al. (1977) reported that *Mucor, Rhizopus* and filamentous yeasts in *bubod*. Kozaki and Uchimura (1990) reported the presence of *Mucor circinelloides, M. grisecyanus, Rhizopus cohnii, Saccharomyces cerevisiae* and *Saccharomycopsis fibuligera* in *bubod*. Sanchez (1986) reported that the molds present in *bubod* ranged from 10^3 to 10^5 cfu/g, yeasts from 10^7 to 10^8 cfu/g, and lactic acid bacteria from 10^5 to 10^7 cfu/g. Hesseltine and Kurtzman (1990) reported that *Saccharomycopsis fibuligera* was dominant in *bubod*. Lim et al. (2006) reported *Sacchromycopsis fibuligera*, *Saccharomyces cerevisiae, Hansunela anomala* from Philippine ethnic amylolytic starter, *bubod* by Genetic DNA Fingerprinting (PCR-RAPD) of yeast isolates.

Chiu-yueh

Chiu-yueh or *peh-yueh* is a gray-white ball-like starter for *lao-chao*, fermented rice product of China. Wei and Jong (1983) isolated yeasts and moulds from *chiu-yueh* and tested the ability of these microorganisms to convert steamed glutinous rice into a good quality *lao-chao.*

Chou or Chu

Chou/Chu is ball, cake or brick (20×22×4.5 cm) shaped and made from moistened raw rice, wheat, sorghum or barley flour (Campbell-Platt 1987). The principal

amylolytic enzyme producers of *chu* are *Rhizopus* and *Mucor* (Yokotsuka 1991). Microbiota in wheat-based *chu* were *Rhizopus japonicus, R. hangchon, R. chinensis, Absidia, Mucor, Monilia, Aspergillus, Lactobacillus* and *Acetobacter* (Otani 1973; Iizuka 1979).

Dhehli

Herbal mix or *dhehli* preparation is an annual community effort, in which elderly people go to forests on the 20th day of Bhadrapada month (usually 5 or 6th September) and collect approximately 36 fresh herbs (Thakur et al. 2004). Some of the important herbs used in *dhehli* preparation are *Pistacia integerrima* (*kkakar shinga*), *Solanum xanthocarpum* (*katari*), *Clitoria ternatea* (*kkayal*), *Aegel marmelos* (*bhel*), *Viola cinerea* (*banaksa*), *Cannabis sativa* (*bhang*), *Trachyspermum copticum* (*ajwain*), *Micromeria biflora* (*chharbara*), *Spiranthes australis* (*bakarshingha*), *Saussurea* sp. (*bbacha*), *Bupleurum lanceolatum* (*nimla*), *Drosera lunata* (*oshtori*), *Salvia sp.* (*kotugha*), *Arisaema helleborifolium* (*chidi ri chun*), *Fragaria sp.* (*dudlukori*). The collected herbs are crushed in stone with a large conical cavity (*ukhal*) using a wooden bar (*mussal*) and the extract as well as the plant biomass are added in to the flour of roasted barley and are roughly kneaded. This is put in to a wooden mould, to give the shape of a brick and dried, is called *dhehli* (Thakur et al. 2004; Savitri and Bhalla 2007).

Daqu

Study of *daqu* Chinese amylolytic starter revealed the presence of filamentous fungal community associated with Chinese wine making process (Chen et al. 2014). *Paecilomyces variotii*, *Aspergillus oryzae* and *Asp. terreus* were reported

from this starter (Chen et al. 2014). The Next generation sequencing (NGS) results of amylolytic starter *daqu* revealed the microbial community including *Saccharomycetaceae* (60%), *Saccharomycopsidaceae* (29%), *Saccharo-mycodaceae* (2%), *Dipodascaceae* (1%), *Trichocomaceae* (< 1%), *Candida* (7%), and *Pleosporaceae* (< 1%) which play an important role during fermentation of *fen,* Chinese rice wine (Li et al. 2011).

Hamei

Hamei is an ethnic amylolytic mixed dry, round to flattened starter of Manipur in India which is very similar to *marcha* (Tamang 2010a). *Hamei* an ethnic amylolytic starter of Manipur is used for the preparation of alcoholic beverage from glutinous rice is very interesting because of its unique flavor and aroma. Yeast communities of *hamei* were identified by phenotypic (biochemical characterization) and molecular tools such as restriction digestion pattern generated from PCR amplified internal transcribed spacer region along with 5.8S rRNA gene (ITS1-5.8S-ITS2) which included yeasts *Saccharomyces cerevisiae*, *Pichia anomala*, *Trichosporon* sp., *Candida tropicalis*, *Pichia guilliermondi*, *Candida parapsilosis*, *Torulaspora delbrueckii*, *Pichia fabianii* and *Candida Montana* (Jeyaram et al. 2008). The genetic diversity of industrially important *S. cerevisiae* group isolated from *hamei* was investigated using Pulsed Field Gel Electrophoresis (PFGE) (Tamang et al. 2007; Jeyaram et al. 2008).

Huamo

Huamo is traditionally prepared rice based ethnic amylolytic starter of Assam and is commonly used for the preparation or fermentation of *judima* (Tamang 2010a). *Humao* is prepared by using locally available glutinous rice, bark, leaves and roots of wild plants (Chakrabarty et al. 2014). During the preparation of *huamo* rice is first washed and powdered in a wooden *okhari along* with the bark, leaves and roots of wild plants parts and few old *humao* starters are mixed properly with clean water to make paste. Then the paste is used to make different sized round to flat, cake-like starters on mat or carpet, fermented for 1-2 days and sun-dried and then stored at room temperature for further use.

Hongqu/yaoqu

Hongqu and *yaoqu* are two popular traditionally prepared amylolytic starters of China (Lv et al. 2012). These traditionally prepared amylolytic starters investigated using a combination of culture-dependent and culture-independent molecular methods. using restriction fragment length polymorphism (PCR-RFLP) analysis of the internal transcribed spacer region ITS1-5.8S-ITS2 and sequencing of the D1/D2 domain of the 26S rRNA gene and generated 12 different genera of yeasts *Pichia, Saccharomyces, Candida, Saccharomycopsis Cryptococcus, Sporobolomyces, Rhodosporidium* and *Rhodotorula* (Lv et al. 2012). On the other hand, the yeast diversity associated with these starters was also investigated through culture-independent method using PCR-DGGE patterns and sequencing of the DNA bands and found almost the same as that of culture-dependent methodology (Lv et al. 2013). The PCR-DGGE fingerprints revealed that *Rhizopus oryzae, R. microsporus* and *Aspergillus* sp. were the most frequent

species in *yaoqu*, while *Monascus sp. dominatedin hongqu* and non-*Saccharomyces* yeasts (*Saccharomycopsis fibuligera, Pichia guilliermondii and Pichiafarinose*) were also detected in some starter samples (Lv et al. 2012). Xu et al. (2012) reported the bacterial DGGE profile targeting the V3 region of the 16S rRNA gene showed that the bacterial composition of starters dominated by *Bacillus* sp., *including B. ginsengihumi, B. megaterium* or *B, aryabhattai, B. subtilis, B. methylotrophicus* and *B. amyloliquefacien* (Xu et al. 2012). Lactic acid bacteria including *Weissella paramesenteroides*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* were also detected in some fermentation starters (Xu et al. 2012). *Rhizopus oryzae, R. microsporus* and *Aspergillus* sp. were the most frequent species in *yao qu,* while *Monascus* sp. dominated in *hong qu* (Xu et al. 2012).

Ipoh

Ipoh is traditionally fermented amylolytic starter of Arunachal Pradesh used for the traditional fermentation of *apong* and *ennog*, popular mild alcoholic beverages (Tamang 2010a). It is prepared through various processes of washing, drying and grinding of glutinous rice into fine powder and mixing powder of leaves, bark, seeds of locally available plant species, viz. *Veronia cinerea* and *Clerodendron viscosum*. Then this mixture is mixed properly in a large container (*dekchi*) and made into paste by using previously stored rice water, spread on clean bamboo mats and made into circular, disc shaped small cake like or biscuit shaped. The cakes are then carefully kept to dry out completely either in the attic above the fireplace of traditional houses or kept in a cool dry place for 4–5 days for fermentation and sun-dried, after drying it is stored for further use. The major

microorganisms involved in *ipoh* are yeast populations (Tiwari and Mahanta 2007).

Khekhri

It a traditionally prepared ethnic unique type starter of Nagaland used to prepare local alcoholic beverage *zutho* (Tamang 2010a). During the traditional preparation, unhulled glutinous rice is washed, soaked into water for 2-5 days, kept and covered with *Khreihenyii* leaves and allowed to germinate for 3-4 days in summer and 5-6 days in winter. After partial germination when the rice sprout is about half inch in length, the sprouted rice is exposed to sun for drying and powdered and again sun dried and stored for further use (Jamir and Rao 1990; Jamir and Deb 2014).

Koji

Koji is mold-culture and is prepared from steamed-cooked cereal (Kitamura 2016). The substrate is usually rice, or sometimes steamed legume beans. The steamed substrate is spread on trays usually made of bamboo strips to depth of 5-7 cm, which are stacked with gaps of about 10 cm in between to allow air circulation. It is followed by inoculation with 0.1 % mold spores, *tane-koji* and incubated at 23-25° C. The rise in temperature due to the growth of mould is kept within the range 35-45° C by stirring and turning *koji* top to bottom on trays at about 20 h and 40 h, normally fermented for 3 days, when mould mycelium spread throughout mass, and before sporulation (Lotong 1985). The mould used is *Aspergillus oryzae*, which is used for starch saccharification in *saké* manufacture (Inoue et al. 1992; Kitamura 2016). Since *koji* is not cultivated in a closed system,

koji is a mixture of several microorganisms. At an early stage of cultivation, yeast grows on steamed rice grain and after that, about 20 h after inoculation of seed *koji*, *koji* mold begins to grow. *Koji* usually contains 10^2/g *saké* yeast, 10^2 to 10^5 g film-foaming yeasts, 10^2 /g lactic acid bacteria, 10^4 to 10^6 /g *micrococci*, 10^7 /g bacilli, etc. Kodama and Yoshizawa (1977) studied the biochemical changes occurring in *koji* and found the increase of reducing sugar from 0.2 % to 21.4 %. Tanaka (1982) studied enzyme activity of steamed or unsteamed glutinous rice-*koji* inoculated with *Aspergillus oryzae* and *Rhizopus jawanicus* and found that α-amylase was 1527 U/g in *Aspergillus* and 100 U/g in *Rhizopus* in steamed rice *koji*, whereas 1255 U/g and 100 U/g in unsteamed rice *koji*, respectively. A combination of *A. oryzae* and *A. sojae* is used in *koji* in Japan to produce alcoholic beverages including *saké* (Zhu and Trampe 2013). *Koji* (Chinese *chu, shi*, or *qu*) also produces amylases that convert starch to fermentable sugars, which are then used for the second stage yeast fermentation to make non-alcoholic fermented soybean *miso* and *shoyu* (Sugawara 2010). *A awamori, A. kawachii, A. oryzae, A. shirousamii*, and *A. sojae* have been widely used as the starter in preparation of *koji* for production of *miso, saké, shoyu, shochu* Suganuma et al. (2007).

Keem

Keem is traditionally prepared barley based ethnic amylolytic starter of Himachal Pradesh and is commonly used for the preparation of *soor* which is commonly consumed as mild alcoholic beverages during various occasions (Rana et al. 2004). During traditional preparation chopped fresh twigs of *Cannabis sativa* (8 kg), 5 kg leaves of *Sapindus mukorossi* and 10-15 kg in total of different plant

species are dried in the shade for few days and then powdered, mixed properly with about 50 kg of barley flour. To the desired quantity of above dry mixture is added a sufficient quantity of Jayaras (a compound prepared by keeping finely cleaved leaves and tender parts of (*Dicliptera roxburghiana*, *Zanthoxylum armatum*, *Leucas lanata* and *Melia azedarach*), in a bigger vessel for overnight night and dough in to a round, circular cake of about 1-2 kg weight. Many oval-shaped cakes are prepared and kept on plant bed (*sathar*) made up of with 15 different tender shoots of *Pinus roxburghii* and *Cannabis sativa* alternately between the cakes incubated in a closed room. The prepared starters are allowed to remain undisturbed for 24 days. On 25th day of incubation, the room is opened and the cake is placed upside down and allowed them to remain there for another 12 days for fermentation. Cakes of *keem* are now taken out, sun-dried, and are used preparation of local alcoholic beverages known as *soor* (Rana et al. 2004).

Loogpang

Loogpang is the starter commonly used in Thailand to prepare alcoholic drink and vinegar (Vachanavinich et al. 1994; Krusong 2014). In *loogpang*, organisms are grown on bran (Steinkraus 1996). The main ingredient of this starter is rice flour with the addition of different type of spices and microorganisms. The microorganisms are originated from the inoculum or surrounding place of preparation of previous batch (Vachanavinich et al. 1994). Pichyangkura and Kulprecha (1977) found that the molds *Amylomyces*, *Rhizopus*, *Aspergillus*, *Mucor*, and *Absidia* in *loogpang*. Dhamcharee (1982) showed that the molds present in *loogpang* from different places in Thailand were *Rhizopus*, *Mucor*, *Amylomyces*, *Penicillium*, and *Aspergillus*, and the main yeast genera were

Endomycopsis (*Saccharomycopsis*), *Hansenula*, and *Saccharomyces*. Sukhumavasi et al. (1975) isolated a strain of *Endomycopsis* (*Saccharomycopsis*) *fibuligera* from *loogpang* with high glucoamylase activity.

Uchimura et al. (1991) reported the presence of *Saccharomycopsis fibuligera* and *Pediococcus* sp. in *loog-pang*. Most studies found *Saccharomycopsis fibuligera* as common yeast in *Loog-Pang* (Limtong et al. 2002). Saelim et al. (2008) reported the Saccharification of cassava starch by *Saccharomycopsis fibuligera* isolated from *Loog-Pang*. Kanlayakrit et al. (1989) Kanlayakrit and Booranasawettatham (2004, 2005) reported that *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* were dominant fungi found in *Loongpang.*

Mod pitha

Mod pitha is traditionally fermented amylolytic starter of Assam and Arunachal Pradesh used for the traditional preparation of *sujen* which is consumed as mild alcoholic beverages (Deori et al. 2007). For the traditional preparation of *mod pitha* glutinous rice grains (*saol*), a handful each of cleaned leaves, fronds, barks, roots and bulb of the various plant parts are put in a round bamboo trey (*saloni*) and exposed for sun drying for a day. The rice grains (*saol*, 4-5kg) is soaked in water for 2 h, cleaned and mixed with the dried plant parts and grounded in a wooden grinder (*dheki*). The grounded rice powder is taken out, sieved in a round bamboo trey (*saloni*) and the coarse part is returned to the wooden grinder (*dheki*) for grinding and this process is continued until a fine powder is obtained. Old *mod pitha* (2 to 3) are added to the mass during grinding. Powdered glutinous rice is put into a utensil (*soriya*), then water is added to make a sticky paste and small round to flattened cakes (2-3 cm in diameter and 0.1 to .04 cm in thickness) are

prepared. Rice cakes are then placed on clean, dry paddy husk spread on a round bamboo trey (*kula*) and again covered with paddy husks. A round bamboo bucket is then kept on a *dhua* sang tied about 1 m above a fireplace in the traditional house kitchen for drying. This procedure of drying the yeasts cake continues for a couple of weeks until this *pitha* becomes harder. *Pitha* is now ready for use in *sujen* fermentation. *Mod pitha* can be stored traditionally for 2-4 months and can be used for the traditional fermentation to make alcoholic beverage *sujen* (Deori et al. 2007).

Malera/treh

Malera/treh is traditionally prepared wheat flour based ethnic amylolytic starter of Himachal Pradesh and is commonly used for the preparation or fermentation of *bhatooru/chilra* which is commonly consumed as staple diet in rural parts of Himachal Pradesh during various occasions (Tamang et al. 2016b). These are prepared with wheat/buckwheat flour dough or slurry fermented with the addition of *malera* which mainly consists of lactic acid bacteria and yeasts (Savitri and Bhalla 2007).

Medombae

There are different types of traditional ethnic amylolytic starters found in Cambodia are *medombae, buh, praa, mesraa, dombae, krrow, paeng* and *poo* (Yamamoto 2016). The starter culture for rice fermentation is known as *medombae* in Cambodia. Spices, herbs, and a sweetener are ingredients commonly added also for dried starter preparation. Water is also added to the mixture and the previous starter was used as a source of inoculum at the rate of 1

to 2%. After mixing thoroughly, the mixture is being shaped into balls manually and placed on layers of rice husks or dried rice straw for 3 days at room temperature, sun-dried, and used as a starter for the production of alcoholic beverages such as rice wine (*Sombai*). Cultural morphological and biochemical identification studied revealed that the isolated representative mold strains were as *Mucor* sp. and *Rhizopus oryzae* and yeasts *Candida tropicalis* and *Saccharomyces cerevisiae* reported by Chay et al. (2017) and Chim et al. (2015).

Mana

Mana is a granular type starter prepared from wheat flakes in Nepal (Tamang 2010a). During its production, wheat grains are soaked in water overnight, steamed for 30 min and is transferred to a bamboo basket, drained and grounded into lump. The floor is cleaned, straw is spread on ground, and wheat lump is placed over it, covered with paddy straw or straw mat, and fermented for 6-7 days. After 7 days, green mold appears on the wheat grains and is dried in the sun to get *mana* and stored. *Mana* contains 10^6 cfu/g of mucorales (*Rhizopus* sp.), 10^7 cfu/g of aspergilla (*Aspergillus oryzae*), 10^3 cfu/g of yeasts and 10^5 cfu/g of LAB (Nikkuni et al. 1996; Shrestha et al. 2002).

Manapu

Manapu is an ethnic amylolytic starter of Nepal similar to *marcha*, which is prepared from rice flour and millets in Nepal (Tamang 2010a). Rice or millet is milled to get flour, and is mixed with 20 % old *manapu*, 5% *manawasha* (white flower of a wild plant), and 5 % black pepper. It is then needed to prepare a cake and placed on straw, which is then covered by straw and fermented at 30-33°C for

5-7 days. Freshly fermented dough is sun dried to get *manapu* microorganisms present in *manapu* are *Saccharomyces cerevisiae, Candia versatilis, Rhizopus* sp. and *P. pentosaceus* (Shrestha et al. 2002).

Marcha

Marcha is a ball-like amylolytic starter, used to ferment starchy materials into fermented beverage in Nepal, Bhutan and the Darjeeling hills and Sikkim in India (Tamang and Sarkar 1995; Tamang 2010a)). During its preparation, glutinous rice is soaked, excess water discarded, pounded, wild herbs, old *marcha* (~1 %) are added, mater thick paste by adding water, and kept on wild fern leaves and fermented for 1-2 days, sun-dried and stored for a year or more (Tamang et al. 1996). Kobayashi et al. (1961) reported *Rhizopus oryzae, Mucor praini* and *Absidia lichtheimi* in *marcha* samples collected from Sikkim. Hesseltine et al. (1988) isolated *Mucor* and *Rhizopus* sp. in *marcha*. Tamang and Sarkar (1995) identified the microorganism in *marcha* of the Darjeeling Hills and Sikkim as *Pediococcus pentosaceus, Saccharomycopsis fibuligera, Pichia anomala, Mucor circinelloides,* and *Rhizopus chinensis.* Batra and Miller (1974) reported *Hansenula anomala* var. *schneggii* (*Pichia anomala)* in *marcha*. In Bhutan, *marcha* is called *chang-poo,* in which *Saccharomycopsis*, *Penicillium* sp. and *Aspergillus* sp. were reported (Uchimura et al. 1990). Microbial profiles of amylolytic starters of India, Nepal, and Bhutan are filamentous molds like, *Mucor circinelloides, Mucor hiemalis, R. chinensis*, and *R. stolonifer* variety *lyococcus* (Tamang et al. 1988); yeasts *S cerevisiae, S bayanus, Saccharomycopsis fibuligera, Sm. capsularis, Pichia anomala, P burtonii*, and *Candida glabrata*;

(Tamang and Sarkar 1995; Shrestha et al. 2002; Tsuyoshi et al. 2005; Tamang et al. 2007; Jeyaram et al. 2011; Tamang et al. 2012).

Nuruk

Nuruk is the starter for preparing Korean alcoholic drink *yakju, takju, makgeolli,* etc. (Jung et al. 2012; Shin et al. 2016). Historically the substrate for *nuruk* was rice but presently it is wheat (Park et al. 1977; Lee and Kim 2016). Generally, *nuruk* is prepared by natural inoculation of molds, bacteria, and yeasts; however, it can be prepared by inoculation with *Aspergillus usamii*. Traditionally *nuruk* is prepared by moistening wheat flour, kneaded and molded into a ball [0.8-1.6 kg (dry weight)] and fermented for 17 days at 30° C to 45° C, dried for 2 weeks and cured for 1-2 months at room temperature (Park et al. 1977). Kim (1968) isolated *Aspergillus oryzae* (10^7 cfu/g), *A. niger* (10^7 cfu/g), *Rhizopus* sp (10^6 cfu/g), anaerobic bacteria (10^7 cfu/g), aerobic bacteria (10^6 to 10^7 cfu/g) and yeasts (10^5 cfu/g) from *nuruk*. Recent advances in high-throughput sequencing technologies such as DNA microarrays and next-generation sequencing (NGS) are rapidly changing the way microbial communities are studied (Roh et al. 2010). The Next Generation Sequencing result represents simple and rapid method of studying microbial ecology that permits the analysis of hundreds of thousands of nucleotide sequences. The phyla *Ascomycota* and *Zygomycota* were the predominant phyla in all samples of *nuruk*, constituting 85.4% (±31.1) and 14.3% (±30.9) of the fungal populations, respectively and *Basidiomycota* at a rate of 0.01%. NGS results of *nuruk*, showed dominance of *Saccharomycopsidaceae, Trichocomaceae, Mucoraceae* and *Saccharomycetaceae* at family level, constituting 99.6% (±0.6) of the fungal sequences (Jung et al. 2012). Yang et al.

(2013) reported that *Aspergillus oryzae* strains isolated from traditional Korean amylolytic starter, *nuruk* improves fermentation properties and rice beverage quality. Bal et al. (2016) identified the dominant *Aspergillus oryzaea* mold from *nuruk* by using molecular (ITS-PCR) and biochemical characterization. They also reported the α-amylase, gluco-amylase as well as acid protease activity. The α-amylase and gluco-amylase activity were higher than the acid protease activity of *Aspergillus oryzaea*. The α-amylase activity was positively correlated with glucoamylase activity. Fungal diversity in wheat-based *nuruk* by NGS and the fungal ITS database, revealed differences in mycobiome composition of the different samples of *nuruk*. Members of both *Ascomycota* and *Zygomycota* dominant in some *nuruk* samples whereas *Zygomycota* dominated some other samples of *nuruk* Bal et al. (2016). In comparison to the domestic samples, the commercial samples dominated by mostly genera of *Pichia*, *Wickerhamomyces*, unclassified members of *Saccharomycetales* Bal et al. (2016).

Phab/dheli

Phab and *dheli* are traditional ethnic amylolytic starters of Himachal Pradesh mostly North West Himalayas used for the preparation of *chhang, jau chhang* and *sura*, alcoholic beverages (Tamang et al. 2016c). The study revealed that yeasts and lactic acid bacteria are the major microflora of these amylolytic starters. Yeasts were identified by sequencing of D1/D2 26S rDNA regions as *Saccharomyces cerevisiae, Saccharomyces fibuligera, Pichia kudriavzevii* and *Candida tropicalis* (Thakur et al. 2015). The dominant lactic acid bacteria (LAB) were *Lactobacillus plantarum, Lactobacillus casei, Pediococcus* and *pentosaceus*

Enterococcus faecium identified on the basis of comparison of the sequence of 16S rRNA genes (Thakur et al. 2015).

Ragi

Ragi is an amylolytic starter culture of Indonesia where rice is used as a substrate (Saono et al. 1974; Surono 2016). During production of *ragi*, mainly rice or millet or cassava or other starchy bases are milled, mixed with herbs and spices, roasted together, sieved, water added and starter (*ragi*) from previous batch is mixed and shaped into balls. These are incubated at 25-30° C for 72 h in humid environment. Balls are dried in the sun and used as inoculum for the various fermentations. Went and Prinsen-Geerligs (1896) found *Monilia javanicus* (*Pichia anomala*) and *Saccharomyces cerevisiae* as principal yeasts in *ragi*. Dwidjoseputro and Wolf (1970) reported the yeasts *Candida parapsilosis, C. melinii, C. lactosa, Hansenula subpelliculosa, H. anomala* and *H. malanga* in *ragi*. Addition of spices to some *ragi* contributes other microorganisms or may inhibit the growth of undesirable microorganisms (Soedarsono 1972). Saono et al. (1974) conducted studies on mycoflora of *ragi* and products fermented by *ragi* such as tape *keté la, tapé ketan hitam, oncom hitam* and *oncom mérah* from various places in West Java and reported that *Candida* sp. was dominating among yeasts, *Mucor* sp. and *Rhizopus* sp. were dominating among moulds. Kato et al. (1976) studied the properties of glucoamylase from *ragi* isolates of *Saccharomycopsis fibuligera*. Saono and Basuki (1978) reported thirteen species of *Candida* from *ragi* of Indonesia. Hadisepoetro et al. (1979) reported that population of yeast in three *ragi* was 5.6×10^6 to 1.4×10^7, bacteria was 3×10^4 to 1.8×10^5 and mould was 3.2×10^4 to 4×10^4. Ardhana and Fleet (1989) reported only single yeast *Candida*

pelliculosa and one mould *Amylomyces rouxii* in four samples of *ragi*. Yokotsuka (1991) reported the presence of mixed cultures in *ragi* mainly *Rhizopus* and *Mucor* among molds; other organisms such as *Amylomyces*, *Aspergillus*, *Fusarium*, *Candida*, *Saccharomyces*, *Hansenula*, *Endomycopsis* (*Saccharomycopsis*). Ishimaru and Nakano (1960) isolated *Streptococcus faecalis, Lactobacillus plantarum* and *Pediococcus pentosaceus* in *ragi* in the range of 10^5 to 10^8 cfu/g. Hesseltine and Ray (1988) reported that most of the bacteria isolated from *ragi* belong to *Pediococcus pentosaceus* and *Streptococcus faecalis*, which may produce secondary products from the glucose formed by the amylolytic yeasts and moulds always found in the starters. Ardhana and Fleet (1989) reported the presence of bacteria in all four samples studied, which included *Bacillus coagulans, B. brevis, B. stearothermophilus* and an unidentified species of *Acetobacter* at the level of 10^3 to 10^4 cfu/g.

Saono et al. (1984) prepared *ragi* by using pure cultures of the selected molds and yeasts, *Amylomyces rouxii* and *Saccharomyces cerevisae* strains and pure culture of *Rhizopus formosaensis* and also prepared brem from this improved *ragi*. Elegado and Fujio (1993) isolated two polygalacturoniase producing strains of *Rhizopus* spp from ragi and studied the enzyme stability in wide range of pH from 2-11 and tolerance at 50º C for 20 min. Uchimura et al. (1991) revealed that there is a higher variability rate of *Pediococcus pentosaceus* in older *ragi* than younger ones and the result suggested that rod-shaped bacteria cannot survive for a long time under dry conditions in *ragi*. Sujaya et al. (2010) reported bacterial diversity of Indonesian *ragi* and their dynamics during the fermentation as investigated by PCR-DGGE the result revealed that lactic acid bacteria were the predominant bacterial flora of *ragi* such as *Pediococcus pentosaceus*, *Enterococcus,*

Lactobcillus sp, *Lactobacillu*s sp., *Enterococcus* sp., *Weissella* sp., and some other bacterial populations were also reported such as *Clostridium perfringent Eubacterium moniliforme, Clostridium sardiniensis,* or *Clostridium baratii Pediococcus, Weissella.* Barus and Steffysia (2013) reported the genetic diversity of yeasts from *Ragi tape* "starter for cassava and glutinous rice fermentation from Indonesia" by using Internal Transcribed Spacer (ITS) region they reported that yeasts *Pichia jadinii* and *Pichia kudriavzevii* are dominant in *Ragi.*

Ranu dabai

Ranu dabai is an amylolytic stater of Assam (Ghosh and Das (2004). During the perpetration of *ranu dabai* six steps are involved: Washing of rice and storing of wash-water. After cleaning then glutinous rice on a *soop,* (a flat traditionally prepared tray generally made up of sliced bamboo) it is taken in a vessel (made of metal/clay) for washing. Clean water is poured in it, mixed and drained off. The discarded wash-water is stored in a container future use. Mixing and grinding: In this step traditional wooden husking machine *dhiki* is used for grinding purpose. The freshly collected plant materials grains are chopped and ground properly and taken out on a *soop*. Glutinous rice is taken in *dhiki* and partially powdered and 3–4 *ranu dabai* large old tablets are added for 10 kg of rice. After some time, paste of various plants is also added to it and mixes properly. The powdered mixture is now taken in a large *dekchi* and made into paste using the previously collected washed rice water. Clean gunny bags are then spread on the floor under shade. These tablets are completely handmade. The standard size is about 4.5–7 cm in diameter, which is kept in rows on the gunny bags, where these tablets are kept for 40–60 min. The sized *ranu* cakes vary from 1.5–15 cm in diameter.

Incubation: It is done inside a large bamboo basket made. Clean and dry straw is spread on the bottom of the bamboo basket and some old *ranu* tablets are kept on it and full basket is covered with the newly prepared *ranu* tablets, after filling of bamboo basket with tablets the basket is covered with polythene sheet or gunny bags and incubated in a dark and warm place and fermented for 2 to 3 days in summer and 4–6 days in winter season. They are taken out from the bamboo basket and are arranged in single layer on circular flat bamboo basket called *dagra* and kept for the sun drying for 7–8 days. After complete drying the *ranu dabai*is ready for storage and for further use for preparation of local alcoholic beverage, *haria* (Ghosh and Das 2004)

Ranu goti

Ranu goti prepared by some ethnic communities of Central India for the preparation of alcoholic beverage such as *handia* (Kumar and Rao 2007). During the perpetration of *ranu goti* firstly the glutinous rice washed, soaked and excess water is drained off and then powdered with help of *dhiki*. The rice powder is now mixed with powdered roots, leaves, bark, rhizomes; seeds of about 20-25 plants species in ratio of 2:1 with clean water and small pieces of cakes were made. These *goti* are kept for incubation in bamboo basket under closed conditions after incubation the *ranu goti* are taken out from the bamboo basket and are exposed to sun for drying for 7–8 days and are used for preparation of local alcoholic beverage, *handia* (Kumar and Rao 2007).

Thiat

Thiat is an amylolytic starter of Meghalaya used to ferment alcoholic beverage–*kiad* (Tamang 2010a). During the preparation of *thiat* firstly the glutinous rice washed, soaked and then powdered. The rice powder is now mixed with powdered, khaw-*iang-/hawiang* plants leaves with clean water and small pieces of cakes were made in size ranging from 4-5 cm in diameter and 0.8-1.0 cm in thickness and are kept for fermentation in *malieng* and covered by *sla-pashor* after fermentation the *thait* are sun-dried and used as dry starters for alcohol production (Samati and Begum 2007).

Vekur pitha

Vekur pitha is traditionally prepared ethnic amylolytic starter of Assam and is commonly used for the traditional preparation of *ahom* which is consumed as mild alcoholic beverages during various ceremonies (Saikia et al. 2007). For the traditional preparation of amylolytic starter, *vekur pitha* glutinous rice grains (*saol*) and leaves of few wild plants are used. The plants ingredients and additive ingredients, which serve as source of, yeast *Saccharomyces cereviceae*. The leaves of plants are collected from the wilderness and exposed to natural sunlight for 2-3 days. Sun dried leaves are powdered and mixed with the powder of rice grain in a vessel containing few ml of clean water. Here, the powder old *pitha* 8 commonly called *ghai pitha* is mixed with freshly prepared *pitha* as source of yeast microflora. The semi-solid *pitha* is mixed with required ingredients and rolled into plate-disc shaped, wrapped with fresh leaves of *Musa paradisiaca* and kept in anaerobic environment over fire heat. The fire heat is maintained at 90-180 cm height for 5-6 days dry till it gets harder. Oval shaped dried *pitha*

containing yeast inoculum, rice powder and plant material is known as *vekur pitha,* which is preserved in natural conditions for future use for preparation of various alcoholic beverages. *Saccharomyces cereviceae* is the major yeast, which plays vital role in fermentation of *vekur pitha* (Saikia et al. 2007).

Xaj-pitha

Bora et al. (2016) reported that *xaj-pitha,* a rice based ethnic amylolytic starter culture of Assam used to prepare the local alcoholic beverages. The microbial community of *xaj-pitha,* by NGS approach revealed the amylase producers, such as *Rhizopus delemar, Mucor circinelloides,* and *Aspergillus* sp. Ethanol producer's viz., *Candida glabrata, Debaryomyces hansenii, Ogataea parapolymorpha, Wickerhamomyces ciferrii, Saccharomyces cerevisiae, Meyerozyma guilliermondii* and *Dekkera bruxellensis* (Bora et al. 2016). Some opportunistic contaminants were also reported from *xaj-pitha*. The bacterial population was dominated by LAB as *Lactobacillus plantarum, Lactobacillus brevis, Weissella cibaria, Lactococcus lactis Leuconostoc lactis, Weissella para mesenteroides, Leuconostoc pseudomesenteroides,* etc. (Bora et al. 2016).

Some Ethnic Alcoholic beverages

Atingba

Atingba is one of the popular traditionally prepared alcoholic beverages of Manipur, prepared from rice (Jeyaram et al. 2009). The Meitei community of Manipur mainly consumes it as food beverage on the several occasions. For preparation of *atingba,* rice is cooked first and then its water is allowed to remove and then cooled to room temperature. The powdered *Hamei* (starter culture for

Atingba) mixed properly with cooked rice with at the ratio of 5 cakes/10 kg of rice. The mixture is then placed within earthen pots, which are covered with *hangla* leaves (*Alocasia* sp.) and then mature is allowed for 3–4 days fermentation in summer and 6–7 days in winter season. This process is then followed by 2–3 days of submerged fermentation in earthen pot to produce the final alcoholic product *atingba*. It is then distilled to give a clear-liquor alcoholic beverage called *yu* in the Manipur. There are various types of yeasts and filamentous fungi that are responsible for fermentation of rice (substrate) to *Atingba* (product) yeasts, *Saccharomyces cerevisiae, C. Pichia anomala, montana, C. parapsilosis, P. guilliermondii, Torulaspora delbrueckii, P. fabianii, Trichosporon* sp., *Candida tropicalis* and molds like *Mucor* sp. and *Rhizopus* sp; whereas some important LAB are *Pediococcus pentosaceus, Lactobacillus brevis* are playing vital role in flavor and texture development (Tamang et al. 2007; Jeyaram et al. 2008).

Bhaati Jaanr

Bhaati jaanr is an ethnic rice-based mild alcoholic food beverage fermented by *marcha* in the Eastern Himalayan regions of Nepa, India and Bhutan (Tamang 2010a). During preparation, first rice is sccharified for 1-2 days in an earthen pot at room temperature and once the saccharification is achieved the vessel is made airtight and is allowed for fermentation for 2-3 days in summer and 7-8 days in winter season. The major microflora involved in *Bhatti Jaanr* saccharification and fermentation are filamentous fungi (*Rhizopus chinensis, M. hiemalis, Mucor circinelloides, R. stolonifer* and var. *lyococcus*) and yeasts (*Candida glabrata, Saccharomyces cerevisiae* and *S. bayanus)*, and Lactic acid bacteria like

(*Pediococcus pentosaceus*, *Lactobacillus bifermentans*, and *Lb. brevis*) (Tamang and Thapa 2006). This microflora is responsible for development of flavor and acidity of the product. pH, titrable acidity, ethanol content and moisture content of the *Bhaati jaanr* is 3.5, 0.24%, 5.9%, and 83.4%, respectively. *Bhaati jaanr* is consumed as a staple food directly in Sikkim and Darjeeling (Tamang 2010a).

Chhang

Chyang or *lugri* is a mild alcoholic, foamy and translucent beverage, which is prepared by traditional fermentation. It is prepared by using the substrate barley (*Hordeum nulum*) locally known as *sherokh* in Ladakh (Bhatia et al. 1977). *Chyang* having a sweet-sour taste and aromatic flavor (Batra and Millner 1976; Batra 1986). During the *chhang* preparation, first Barley grains are cooked over a slow fire in the water just sufficient for absorption it and then after cooking the mixture is spread on blanket or burlap mat to remove the access water. The cooked barley grains at lukewarm stage are mixed with starter culture, *phab* using in ratio of 1g/kg of barley. These mixtures are filled in drill bags, mostly in 20-kg batches, and then tightly packed. These mixtures are then packed by gunny bags to maintain the temperature around 30°C–35°C which is required for fermentation of barley it to *Chyang* after 7–8 days of fermentation (Bhatia et al. 1977). Microorganisms that plays significant role in the fermentation process of *Chhang* are yeasts *Saccharomyces cerevisiae* and *S. uvarum* (Batra 1986). *Chyang* is one of the popular mild alcoholic beverages traditionally prepared and consumed by the people of Ladakh (Bhatia et al. 1977).

Kodo ko Jaanr

Kodo ko jaanr is one of the most popular ethnic fermented finger millet (*Eleusine coracana*) beverages of the Himalayan regions of India with mild-alcoholic (4.8 %) and sweet taste (Tamang 2010a). *Kodo ko jaanr* has several synonyms as used by different ethnic groups of the Himalayan people such as *chyang* (Tibetan, Ladakhi, Drupka), *mandokpenaa thee* (Limboo), *mong chee* (Lepcha) (Tamang et al. 2016b). During its production, finger millet seeds are cleaned, washed and cooked for about 30 min, excess water is drained off and cooked millets are spread on a bamboo mat for cooling. About 1-2 % of powdered *marcha* is sprinkled over the cooked seeds, mixed thoroughly and packed in a bamboo basket lined with fresh fern (*Thelypteris erubescens*) and then covered with sack cloths, and fermented at room temperature for 2-4 days. The saccharified mass is transferred into an earthen pot or bamboo basket, made air-tight and fermented for 3-4 days during summer and 5-7 days in winter at room temperature for alcohol production. Freshly fermented *kodo ko jaanr* is filled into a bamboo-made vessel locally called *toongbaa*, and lukewarm water is added up to its edge and leave it for 10-15 min. Then, the milky white extract of *jaanr* is sipped through a narrow bamboo straw called *pipsing* which has a hole in a side near the bottom to avoid passing of grits. Water is added twice or thrice after sipping of the extract. Consumption of fermented finger millet beverages in exclusively decorated bamboo or wood-made vessel called *toongbaa* is unique in the Himalayas (Tamang et al. 1996). *Kodo ko jaanr* liquor is believed to be good tonic for ailing persons and post-natal women. After consumption, residual or grits of *kodo ko jaanr* are used as fodder for pigs and cattle. This is a good example of total

utilization of substrate as food and fodder, and also the discarded grits contain nutrient used as animal feed.

Marcha used as amylolytic starter supplements all functional microorganisms in *kodo ko jaanr* fermentation (Thapa and Tamang 2004). Mycelial molds have roles only in the initial phase of fermentation mostly in saccharification of the substrates. Yeasts *Pichia anomala, Saccharomyces cerevisiae, Candida glabrata, Saccharomycopsis fibuligera*, and LAB *Pediococcus pentosaceus* and *Lactobacillus bifermentans* have been recovered in *kodo ko jaanr* samples. Population of filamentous molds, which were originated from *marcha,* declines daily during *in situ* fermentation of *kodo ko jaanr* and finally disappears after fifth day (Thapa and Tamang 2006). *Sm. fibuligera* and *R. chinensis* saccharify and liquefy millets starch into glucose and produce alcohol *in situ* fermentation of *kodo ko jaanr*. Fermentation of finger millet enhances bio-enrichment of minerals such as Ca, Mg, Mn, Fe, K, P, contributing to mineral intake in daily diet of rural people (Thapa and Tamang 2004). Ailing persons and post-natal women consume the extract of *kodo ko jaanr* to regain the strength due to high calorie in *jaanr.*

Sujen

It is a mild alcoholic beverage is popular among the Deori, an ethnic community of Assam (Deori et al. 2007). It is also considered as pure and used as a holy water by the Deoro priests during various festivals and ceremonies. During *sujen* preparation, first the preparation of the natural starter called *mod pitha* is done and then the fermentation of *sujen* (Deori et al. 2007). Several types of plants species used for the preparation of *mod pitha* starter. Five kg of glutinous rice is soaked for about 2 hours in water, cleaned then mixed properly with the dried plant parts in a grounded in

dheki, a wooden grinder along with old *mod pithas* starters. The grounded starter powder is taken in a vessel for fermentation for duration of 7-15 days. After fermentation it is diluted for consumption (Das et al. 2012).

Lao-Chao

Lao-Chao is one of the famous alcoholic fermented beverages of China (Steinkraus 1996). During preparation, rice is boiled and then allows it for cooling on a mat, and then mixed properly with yeast cultures grown on rice and nosan leave. The yeast inoculated rice is then poured into a cone-shaped bamboo basket and an earthen pot is placed under the cone for the collection of the liquefied rice as it ferments. The fermented product (juice) is collected and transferred to new boiled rice for about 3 or 4 times in succession. The dominant microorganisms consists of filamentous fungi mainly *Rhizopus*, *Mucor,* yeasts and lactic acid bacteria. The final alcohol content of the product ranges from 12 to 14% (v/v) with pH 3.9 (Wang and Hesseltine 1970; Wei and Jong 1983).

Poko

Poko is traditionally prepared rice fermented alcoholic beverage of Nepal (Shrestha et al. 2002). It is very similar to *Bhatii ko Jaanr* an alcoholic beverage of Sikkim and Darjeeling Himalayas. It is generally consumed and served during the festive seasons and various ceremonies by the people of Nepal. The dominant micro-biota which plays important role during fermentation of *poko* are mainly *Rhizopus* and yeasts like *Saccharomyces cerevisiae, Candida versatile* and lactic acid bacteria, *Pediococcus pentosaceus* also playing very significant role in the product and flavor development.

This traditionally prepared ethnic alcoholic beverage of Nepal has strong socio-cultural significance (Shrestha et al. 2002).

Tapé ketan

Tapé ketan is a traditionally fermented, sweet/sour, alcoholic beverage of Indonesia (Steinkraus 1996). The cassava (*tapé ketella*) and glutinous rice (*tapé ketan*) are most common substrate used for *tapé ketan* fermentation. During preparation of *tapé ketan* the glutinous rice is washed and soaked for 1 h in water then cooked well, spread over a bamboo tray and then allowed to cool to room temperature. Then powdered *ragi*, amylolytic starter culture is sprinkled and mixed properly with rice and then placed in an earthenware pot for traditional fermentation. The sticky rice is converted to a soft, juicy mass with a sweet/sour; alcoholic flavor within 2 to 3 days of fermentation at room temperature now the product is ready for consumption. The *Tapé ketan* is acceptable for consumption even after one week of fermentation (Cronk et al. 1977). With the long fermentation the product becomes more liquid. The product gets ready for consumption after 36 to 48 h of fermentation at 30°C (Cronk et al. 1977). Malaysian *tapai* is also alcoholic beverage contains 27% of total sugar, 5% of ethanol (v/v), 23% of reducing sugar and pH of the product is 3.9 is acidic (Steinkraus 1996). *Tapé ketan* must be sweet to be edible and acceptable hence, the final product must be consumed between 3 to 4 days when the content of the reducing sugars in the product are highest (Merican and Yeoh 1977).

Saké

Saké is a national drink of Japan and is one of the most popular traditional non-distilled alcoholic drinks in the world (Jin et al. 2005). It is prepared from rice using *koji* and is clear, pale yellow, containing 15 to 20 % alcohol. Polished rice is washed, steeped in water and steamed for 30-60 min, and then cooled, mixed with *koji*, water and a selected yeast starter culture for alcoholic fermentation. Main fermentation takes place in open tanks in cool conditions, starting at about 10° C, increasing to about 15°C. After fermentation, the liquid material called *moromi* is separated from the solids to give the clarified *saké*, which is settled, re-filtered, pasteurized and blended and diluted with water before bottling (Yoshizawa and Ishikawa 1989). Unique strains of *S. cerevisiae* have evolved to conduct those fermentations generating products with high ethanol content (12-20%), attractive flavor and aroma and odor (Kodama 1993). The first organisms developed in the mash under traditional fermentation conditions are nitrate-reducing bacteria such as *Pseudomonas, Achromobacter, Flavobacterium,* or *Micrococcus* spp. (Murakami 1972). These are followed by *Leuconostoc mesenteroides* variety *saké* and *Lactobacillus saké* and yeasts (Kodama and Yoshizawa 1977). The highly refined *saké* brewed by the most skillful brewers using very highly polished rice at low temperatures of 9 to 11°C for 25 to 30 days is known as *gonjoshu* (Kodama and Yoshizawa 1977). Most LAB that spoil *saké* are homofermentative rods and are more tolerant to ethanol and acid than non-spoilers (Inoue et al. 1992).

Difference in responses to osmotic stress between the laboratory and *saké*-brewing strains of *Saccharomyces cerevisiae* at the translational level was compared and found that enhancement of glycerol formation due to enhancement of the translation of proteins Hor2p, is required for growth of *S. cerevisiae* under high osmotic pressure

condition (Hirasawa et al. 2009). *Saccharomyces cerevisiae* strains with disrupted ubiquitin-related genes produced more ethanol than the parental strain during *saké* brewing (Wu et al. 2009). Several researchers have reported on improved strains of *Aspergillus oryzae* for *saké* production in industrial scale (Hirooka et al. 2005; Kotaka et al. 2008; Hirasawa et al. 2009).

Tapuy

It is a highly acidic but alcoholic, sweet, aromatic and flavored rice beverage of Philippines (Steinkraus 1996). It is also known by other names as. In the process of preparation of *tapuy*, glutinous or ordinary white rice or a mixture of the two is soaked, cleaned then ground in a stone mill. The mash is mixed with pureed ginger and/or wild herbal root and starter culture, *bubod* from previous batches, incubated for three days, and dried (Sakai and Caldo 1983). *Saccharomycopsis fibuligera*, *Saccharomyces uvarum* is the major yeast flora playing vital role during fermentation of the *tapuy* (Sakai and Caldo 1985). Sakai and Caldo (1985) were reported that the enzyme glucoamylases were the primary amylases produced by *S. burtonii, S. fibuliger,* and Mucor molds helping in saccharification (conversion of polysaccharides to monosaccharide's) fermentation as well as product and flavor development. The ethanol concentration of the final product is 4.93 %(v/v) on day 2 of fermentation and reached up to level of 15.5% v/v on day 14 of fermentation. Sanchez et al. (1985) reported that eight different varieties of *bubod,* yielded 12.9 to 17.3% (v/v) of ethanol in *tapuy,* with final pH of 3.9 to 4.5.

Zutho

Zutho is a mild alcoholic beverage popular among the Mao community in Nagaland (Tamang 2010a). In the preparation of *zutho,* firstly the rice is washed, soaked in water overnight, water is drained off, grinded in to powdery form and this is put in to bamboo bucket and mixed properly with warm water, then allow it for cooling, after cooling the powdered amylolytic starter which is locally known as *khekhrii* (Mao and Odyuon 2007) mixed properly and brewed for 7-8 h. After proper mixing the whole mass is poured in to earthen pot and more fresh water is added up to neck. Now this earthen pot kept for 3-4 days fermentation (Mao 1998). *Nchiangne* is another similar alcoholic beverage is prepared from glutinous rice in Nagaland (Tamang et al. 2012). The physiochemical profile of *zutho* showed the pH of the product is about 3.6, alcohol contents 5.1% and acidity of 5% Teramoto et al. (2002).

MATERIALS AND METHODS

MEDIA USED

(1) Sugar fermentation

Fermentation Basal Medium (FBM)	(Yarrow 1998)
Yeast extract powder	4.5 g
Peptone	7.5 g
Distilled water	1 litre/1000 ml
Bromothymol blue stock solution	
Bromothymol blue	50 mg
Distilled water	75 ml

(Add 4 ml of the stock solution per 100 ml of fermentation basal medium)

(2) Sugar assimilation	(Yarrow 1998)
Yeast nitrogen base (YNB)	6.7 g
Sugar	5%
Demineralised water	100 ml

(3) Starch Agar	(Gordon et al. 1973)
Starch	10% (w/v)
Trypyone	50 g
Yeast extract powder	15.0 g
Potassium dihydrogen phosphate	3.0 g
Agar	20.0 g
Distilled water	1 litre/ 1000 ml

(4) Nitrate reduction test

Nitrate broth	(Gordon et al. 1973)
Peptone	5.0 g
Beef extract	3.0 g
Potassium nitrate	1 g
Distilled water	1 litre/ 1000ml
pH	7
Zinc Powder	0.3g

(5) Yeast- Malt Agar (YMA)	(M424, Himedia, Mumbai)
Yeast Malt Agar	41 g
Distilled water	1 litre

(6)Yeast-Malt Broth (YMB)	(M425, Hi media, Mumbai)
Yeast Malt Broth	21.0 g
Distilled water	1 litre

(7) Malt-Extract Agar	(M137, Hi media, Mumbai)
Malt Extract agar	50.0 g
Distilled water	1 litre

(8) Potato Dextrose Agar (PDA)	(M096, Hi media, Mumbai)
Potato dextrose agar	39.0 g
Distilled water	1 litre

(9) Ascospore Agar (M804, Hi media, Mumbai)

Ascospore agar	43.5 g
Distilled water	1 litre

(10) Sugars

Arabinose	(RM 045, Himedia, Mumbai)
Cellobiose	(RM 098, Himedia, Mumbai)
Dextrose (glucose)	(RM 077, Himedia, Mumbai)
Galactose	(RM 101, Himedia, Mumbai)
Glycerol	(RM 101, Himedia, Mumbai)
Inositol	(RM 102, Himedia, Mumbai)
Lactose	(RM 565, Himedia, Mumbai)
Maltose	(RM 018, Himedia, Mumbai)
Melibiose	(RM 106, Himedia, Mumbai)
Mannitol	(PT0604, Himedia, Mumbai)
Raffinose	(RM 107, Himedia, Mumbai)
Rhamnose	(RM 062, Himedia, Mumbai)
Starch	(RM 089, Himedia, Mumbai)
Sucrose	(RM201, Himedia, Mumbai)
Trehalose	(RM 110, Himedia, Mumbai)
Xylose	(RM 111, Himedia, Mumbai)

(11) Ascospore Agar	(M804, HiMedia, Mumbai)
(12) Fermentation Basal Medium for yeasts	(Wickerham, 1951)
(13) Malt Extract Agar	(M137, HiMedia, Mumbai)
(14) MRS Agar	(M641, HiMedia, Mumbai)
(15) MRS Broth	(M369, HiMedia, Mumbai)
(16) Nitrate Broth	(Gordon et al. 1973)
(17) Nutrient Agar	(MM012, HiMedia, Mumbai)
(18) Nutrient Broth	(M002, HiMedia, Mumbai)
(19) Plate Count Agar	(M091, HiMedia, Mumbai)
(20) Sucrose Broth	(Garvie 1960)
(21) Yeast-Malt Extract Agar	(M424, HiMedia, Mumbai)
(22) Yeast Malt Extract Broth	(M425, HiMedia, Mumbai)
(23) Yeast Morphology Agar	(M138, HiMedia, Mumbai)
(24) Yeast Nitrogen Base	(M139, HiMedia, Mumbai)
(25) Potato Dextrose Agar	(M096, HiMedia, Mumbai)
(26) Ethidium bromide	(RM813, Himedia, Mumbai)

REAGENTS USED

(1) Nitrate reduction test reagent (M439S, Himedia, Mumbai)

Solution A

Sulphanilic acid	0.8 g
5 N Acetic acid	100 ml

Solution B

α- Napthylamine	100 ml
5 N Acetic acid	100 ml

(If acetic acid is not present instead glacial acetic acid can be used in following way (Glacial acetic acid: water = 1: 2.5).
(The two solutions A and B were mixed in equal quantities just before use).

(2) Safranin	(RM1315, Himedia, Mumbai)
Safranin	2.5 g
95% ethanol	100 ml
(3) Malachite green (5% solution)	(S020, Himedia, Mumbai)
Malachite green	5.0 g
Distilled water	100 ml
(4) Iodine solution	(M425, Himedia, Mumbai)
Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	300 ml
(5) Acidic Ninhydrin	(RM248 Himedia, Mumbai)
1-Butanol/water saturated	465 ml
Acetic acid	35 ml
Ninhydrin	2.5 ml
(6) Lugals Iodine Solution	(S019, HiMedia, Mumbai)
Iodine	2.0 g
Ammonium Sulphate	2.0 g
Distilled water	300 ml

(7) Gram's Crystal Violet	(S012, HiMedia, Mumbai)
(8) Malachite Green	(S020, HiMedia, Mumbai)
(9) Nessler's Reagent	(R010, HiMedia, Mumbai)
(10) DNA extraction kit	(TM050, ProMega, USA)
(11) PCR- Gel Purification kit	(A9281, ProMega, USA)
(14) Forward ITS1 and reverse primer ITS4	(C1181, ProMega, USA)
(15) 8% Polyacrylamide gels	(Promega, V3111, USA)
(16) 1×TAE buffer	(ML016, HiMedia, Mumbai)
(17) Phenolphthalein	(I009-125ML, HiMedia, Mumbai)
(18) Agarose	(V3125, Promega, US)
(19) Methyl red	(I007, Himedia, Mumbai)
(20) Oxalic acid	(MLOM, Merck Millipore, US)
(21) Ethanol	(MB106, Himedia, Mumbai))
(22) Gel loading dye	(G1881, Promega, US)
(23) Urea Pure	(208884,Sigma-Aldrich, US)
(24) Formamide	(MB012, Himedia, Mumbai)
(25) SYBER Gold	(S9430, Promega, USA)
(26) Ammonium per Sulphate-APS	(MB003, Himedia, Mumbai)
(27) Nuclease free Water	(P1193, Promega, USA)
(28) Tetramethylethylenediamine	(5965-833, Himedia, Mumbai)
(29) Sodium Hydroxide Solution	(MF8D, Merck Millipore, US).
(30) Gotaq green Master Mix	(M7122, Promega, US)
(31) Proteinase K	(V3021, Promega, US)

(32) RNAase	(A7973, Promega, US)
(33) DNSA	(GRM1582, Himedia, Mumbai)
(34) Phenol	(MB082, Himedia, Mumbai)
(35) Ferric Sulphate	(FD237, Himedia, Mumbai)
(36) Primer (NL1 and LS2)	(C1101, ProMega, USA)
(37) GC clamp 30 bp sequence	(C1101, ProMega, USA)

INSTRUMENT USED

(1) Phase contrast microscope	(Olympus, CKX41, Japan)
(2) Compound Microscope	(Olympus, EX1000, Japan)
(3) Biological Incubator	(Accumax, CIS-24BL, Kolkata)
(4) Water Distillation unit	(Riviera, 72240020, Kolkata)
(5) Mechanical oven	(Instrumentation India, Kolkata)
(6) Laboratory Autoclave	(Instrumentation India, Kolkata)
(7) Orbital Shaker Incubator	(Remi, RSB-12, Mumbai)
(8) -80 Freezer Vertical	(TSE240A, Thermo fisher, USA)
(9) -20 Freezer	(Remi, ROFV-170, Mumbai)
(10) Desiccator	(DURAN, DIN-12491, USA).
(11) Water bath Shaker	(Digilab, EX9UA, Mumbai)
(12) High precision water bath	(Remi, RIME-1322, Mumbai)
(13) Magnetic stirrer	(Remi, 2MLH, Mumbai)
(14) Centrifuge	(Thermo Scientific, CL21, USA)
(15) Digital PH meter	(Thermo Scientific, A321, USA)
(16) Analytical weighing balance	(Mettler, AX 204 Kolkata)
(17) Microwave	(Samsung, 28L Mumbai)
(18) Anaerobic gas pack system	(HiMedia, LE002, Mumbai)
(19) Vertical Laminar Air flow	(Thermo Scientific, 1386, USA)
(20) UV-Transilluminator	(Remi, E3000 UV, Mumbai)
(21) Gel-documentation Unit	(Bio Rad, 97-0186-02, US)
(22) Electrophoresis Unit	(Remi, R-24, Mumbai)
(23) ABI-DNA-Sequencer	(ABI 3500, HITACHI, Japan)
(24) NGS Illumina-Miseq	(Illumina platform, USA)
(25) Nano-DropND-1000	(Nano-Drop technologies, 1000, USA)
(26) Qubit Fluorimeter	(Invitrogen, Q33227, USA)
(27)Thermal Cyclers	(Applied Biosystems-2720, USA)

(28) Sequence Scanner	(Applied Biosystems-V1.0, USA)
(29) SEQMANN software	(DNASTAR, 4462914, USA)
(30) Spectrophotometer	(Perkin-Elmer, LAMBDA 950**,** USA)
(31) DCode™ Universal Mutation Detection System	(DGGEK-1001, CBS-Scientific, USA)
(32) Biolog Identification System	(MicroLog TM System Release 4.2 User Guide 2001, Biolog Inc.)

METHODOLOGY

SURVEY

A extensive field survey was conducted in different villages and local markets of eight states of North-East India namely viz; Sikkim, Meghalaya, Assam, Arunachal Pradesh, Manipur, Mizoram, Tripura and Nagaland and sought the information on traditional methods of preparation, their uses for productions of alcoholic beverages and socio-economy of ethnic fermented amylolytic starters. The documentation was carried during 2014 to 2016. Data collection was done based on structured questionnaire, interviewing the ethnic people practicing traditional knowledge of preparation of amylolytic starters, personally analyzing the preparation procedures, collection of plant parts used during the preparation which were identified with the help of Botanical Survey of India, Sikkim branch.

COLLECTION OF SAMPLES

A total forty different sun-dried traditionally prepared amylolytic starter samples (5 samples of each starter) *marcha* of Sikkim, *thiat* of Meghalaya, *hamei* of Manipur, *phut* of Arunachal Pradesh, *chowan* of Tripura, *dowdim* of Mizoram, *humao* of Assam and *khekhrii* of Nagaland were collected immediately after the preparation (fermentation and sun-dried drying) from local people of different parts of North East states in India, respectively. Sealed gamma irradiated sterile bottles were used for collection of sample and then samples stored in desiccator at room temperature for the further analysis. Traditionally prepared starter retains its potency *in situ* for over a year or more in moist-free condition at room temperature (Tamang and Sarkar 1995); hence samples were kept at desiccators at room temperature in laboratory for further analysis.

CULTURE-DEPENDENT ANALYSIS

Phenotypic characterizations

Isolation microorganism

Ten gram of sample was homogenized with 90 ml of 0.85% (w/v) sterile physiological saline in a stomacher lab-blender 400 (Seward, UK) for 1 min and serially diluted in the same diluents. Yeasts and molds were isolated on potato dextrose agar (M096, HiMedia, India) and yeast-malt extract agar (M424, HiMedia, India), respectively supplemented with 10 IU ml^{-1} benzyl-penicillin and 12 mg ml^{-1} streptomycin sulphate and incubated aerobically at 28°C for 3 days. Purity of the isolates was checked by streaking again on fresh agar plates of the same isolation medium, followed by microscopic examination. Colonies appeared were counted as colony forming units (cfu)/g sample. Identified strains of yeasts were preserved in 20% glycerol at -20°C (Thapa and Tamang 2004).

Cell morphology

Cell morphology and mode of vegetative reproduction of yeast was observed following the method of Yarrow (1998). Sterile yeast morphology agar (M138, HiMedia, and Mumbai) slants were inoculated with an actively growing (24 hour-old) yeast culture and incubated at 28°C for 3 days. Cell morphology of yeast isolates was determined using a phase contrast microscope (CH3-BH-PC; Olympus, Tokyo, Japan).

Pseudo- and True-mycelium

For observation of pseudo-mycelium and true-mycelium of yeast isolates, the slide culture method described by Kreger-van Rij (1984) and (Yarrow 1998) was followed. A Petri-dish, containing U-shaped glass rod supporting two glass slides, was autoclaved at 121°C for 20 min. The glass slides were quickly removed from the glass rod with a flame sterilized pair of tweezers, and were dipped into the molten potato dextrose agar (M096, HiMedia, Mumbai) after which they were replaced on the glass rod support. The solidified agar on the slides was inoculated very lightly with yeast isolates in two lines along each slide. Four sterile cover-slips were placed over part of the lines. Some sterile water was poured into the Petri-dish to prevent the agar from drying out. The culture was then incubated at 28°C for 4 days. The slides were taken out of the Petri-dish and the agar was wiped off from the back of the slide. The edges of the streak under and around the cover-slips were examined microscopically for the formation of pseudo-mycelium or true-mycelium.

Characteristics of ascus and ascospore

Sterile ascospore agar (M804, HiMedia, and Mumbai) slants were streaked with actively grown yeast cultures, incubated at 28°C for 3 days and examined at weekly intervals up to 4 weeks for observation of asci and ascospores. A heat fixed smear was flooded with 5 % w/v aqueous malachite green (S020, HiMedia, Mumbai) for 30 to 60 sec, heated to steaming 3 to 4 times over the flame of a spirit lamp and counterstained with safranin (S027, HiMedia, Mumbai) for 30 sec and observed under the microscope (Yarrow 1998).

Reduction of nitrate

Yeast cultures were grown in 5 ml nitrate broth incubated at 28°C. After 3, 7 and 14 days, 1 ml of the culture was mixed with 3 drops of the reagent for nitrate reduction test and observed for the development of a red or yellow color, indicating the presence of nitrate. A small amount of zinc dust was added to the tube that was negative even after 14 days and observed for the development of red color, indicating the presence of nitrate, i.e. absence of reduction (Yarrow 1998).

Growth at 37°C

Slants of malt-extract agar (M137, HiMedia, and Mumbai) were inoculated with cells of actively grown yeast isolates and incubated at 37°C for 4 days and observed for growth (Yarrow 1998).

Growth at 45°C

Slants of malt-extract agar (M137, HiMedia, and Mumbai) were inoculated with cells of actively grown yeast isolates and incubated at 37°C for 4 days and observed for growth (Yarrow 1998).

Sugar fermentation

Yeasts isolates were grown at 28°C on yeast-malt extract (YM) agar (M242, HiMedia, Mumbai) slants for 3 days. Tubes of 10 ml of fermentation basal medium (Wickerham 1951) supplemented with 2 % w/v sterile sugars inoculated with the above yeast culture and incubated at 28°C and were shaken to observe (Yarrow 1998).

Sugar assimilation

The Yeast isolates were grown at 28°C on yeast-malt extract (YM) agar (M242, HiMedia, Mumbai) slants for 3 days. Tubes containing 5 ml mixture of yeast nitrogen base (M139, HiMedia, Mumbai) and carbon source were inoculated with cultures and incubated at 28º C for 3 to 7 days. Control test tube was made by adding 0.5 ml of yeast nitrogen base in 4.5 ml of sterilized distilled water (devoid of any carbon source). Assimilation of carbon sources was observed by comparing with the control (Yarrow, 1998) Yeast isolates were identified to the genus level according to the criteria laid down by Kurtzman et al. (2011) and Yarrow (1998).

Biolog identification system

The phenotypic identification of yeast isolates were done by using Biolog Identification System (MicroLog TM System Release 4.2 User Guide 2001, Biolog Inc.) based on the utilization of 95 substrates in 96 welled plate, were used for biochemical characterization of yeast isolates. Aliquots of the cultures were transferred to biolog plate wells and incubated at 37°C for 24-48 h, where positive results were recorded according to colour changes. The results obtained were automatically give a specific metabolic fingerprint and analysed using Biolog Microlog Reader and compared with the database of the Biolog software (Biolog Inc), which provided the closest genera and species of the tested isolates.

Molecular Identification of Yeast Isolates

DNA extraction and PCR-amplification

Yeast DNA was extracted using ProMega DNA kit (ProMega). One gram of yeast cell pellet 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 centrifugation 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 (NanoDrop technologies, Willington, USA) as described by Kumbhare et al. (2015). The DNA was stored at -20°C until further processing.

ITS-PCR

For the amplification of the Internal Transcribed Spacer (ITS) region, the forward primer ITS1 (5'-NNNN-3') and reverse primer ITS4 (5'-NNNN-3') (White et al. 1990) were used and PCR mixture and the thermal cycling protocol conditions were applied. The PCR reactions were run for 30 cycles at 94°C for 60 sec for denaturation, at 50°C for 30 sec for annealing, and at 72°C for 60 sec the final extension for 6 min at 72°C as described by Esteve-Zarzoso et al. (1999). Products were analyzed on 1.5% agarose gel containing 0.7 mg/ml of ethidium bromide and visualized under UV light (UV source Gel-Doc 1000, Bio-Rad). Approximate size of amplified products was determined using standard molecular weight markers (Himedia-100-bp DNA Ladder) (Lv et al. 2013). All PCR-amplified products were purified and sequenced using ABI-DNA-Sequencer (ABI Genetic Analyser 3500, HITACHI, Japan). The

sequences were compared with the GenBank database using the BLAST programme (Altschulet al. 1990; Zhao et al. 2014). Sequences were visualized and edited using Chromas Version 1.45 (http://www.technelysium.com.au/chromas.html) (Pryce et al. 2003).

CULTURE INDEPENDENT TECHNIQUE

PCR-DGGE analysis

Genomic DNA extraction and *PCR amplification*

For culture independent technique genomic DNA was directly extracted from sample. About 10 g of samples was homogenized in 90 ml of 0.85% w/v sterile physiological saline, and subsequently filtered through 4 layers of sterile cheese-cloth. The resulting filtered solutions were centrifuged at 14,000 g for 10 min at 4°C (Lv et al. 2013). Then the pellets were subjected to DNA extraction using the ProMega DNA extraction kit (ProMega, USA) according to the manufacturer's instructions. Quality of resultant DNA was checked on 0.8% agarose gel and concentration was measured Nano-DropND-1000 spectrophotometer (NanoDrop technologies, Willington, USA) as described by Kumbhare et al. (2015).

The 250 nucleotides of the 5′- end D1/D2 region of the 26SrRNA gene was amplified by PCR using the primer NL1 (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC ATA TCA ATA AGC GGA GGA AAA G-3′) (the GC clamp sequence used is underlined) and a reverse primer LS2 (5′-ATT CCC AAA CAA CTC GAC TC-3′) (Cocolinet al. 2000; El Sheikha et al. 2009). PCR was performed in a final volume of 50µl containing 10 mMTris–HCl, 50mM KCl, 1.5mM $MgCl_2$, 0.2mM each dATP, dCTP, dGTP and dTTP, 0.2mM of the primers, and 1.25IU Taq-DNA polymerase (Promega, USA) and 2µl of the extracted DNA (approximately

50ng) using Thermal Cyclers (Applied Biosystems, USA). The reactions were run for 30 cycles at 95°C for 60 sec for denaturation, at 52°C for 45 sec for annealing, and at 72°C for 60 sec for extension and finally for 7 min at 72°C (Cocolinet al. 2002). The PCR products were analysed on 2.0% agarose gel containing 0.5µg/ml ethidium bromide and were visualized in UV light (UV source Gel-Doc 1000, Bio-Rad) (Cocolin et al. 2000). The concentration was again measured using Nano-DropND-1000 spectrophotometer (NanoDrop technologies, Willington, USA).

PCR-DGGE

The Polymerase Chain Reaction (PCR) products were analyzed by denaturing gradient gel electrophoresis (DGGE) using DCode™ Universal Mutation Detection System (DGGEK-1001, CBS-Scientific, San Diego, USA) following the procedure of El Sheikha et al. (2009). Samples containing approximately equal amounts of PCR products were loaded into 8% w/v polyacrylamide gels (acrylamide:*N*,*N*′-methylenebisacrylamide, 37.5:1; Promega) in 1×TAE buffer (40 mM Tris–HCl, pH 7.4, 20mM sodium acetate, 1.0mM Na_2-EDTA). All electrophoresis experiments were performed at 60°C using a denaturing gradient in the range of 30–50% (100% corresponded to 7M urea and 40% v/v formamide; Promega) (Cocolinet al. 2002). The gels were electrophoresed at 20 V for 10 min and then at 80 V for 12 h (El Sheikha et al. 2009). The gels were stained with SYBR Gold for 30 min (reconstituted according to the manufacturer's directions; Molecular Probes, Invitrogen, USA) and photographed in UV light (UV source Gel-Doc 1000, Bio-Rad) as described by Grizard et al. (2014). The DGGE bands were excised using sterile micro pipette tips. DNA of each band was eluted in 50µl sterile water overnight at 4°C and 2µl of the eluted DNA was reamplified as following the method of Cocolin et al. (2000). The

PCR products which yielded only one band in DGGE electrophoresis were amplified with the primers without GC-clamp, purified and finally sequenced with the help of ABI-DNA-Sequencer (ABI Genetic Analyser 3500, HITACHI, Japan). The sequences were compared with the GenBank database using the BLAST programme (Altschul et al. 1990; Zhao et al. 2014). The DNA sequences obtained from sequencing of total 203 bands was submitted to Gene Bank.

Firstly 0.6 volumes of 20% PEG-NaCl added to the final volume of PCR product and then Incubated at 37°C for 20-30 min then centrifugation of the product was done at 12,000/3800 rpm for 30 min after the centrifugation of the PCR product the supernatant was decanted and centrifugation tube was vortexed at 400 rpm for 5-10 seconds. In the pellet 100 µl 70% ethanol was added again centrifuged at 12,000/3800 rpm for 30 min again decant/Invert spin at 400rpm for 5-10 seconds then air dry and then add 12µl distilled water and finally purity of the PCR product was check on 1% Agarose gel electrophoresis (Zhao 2014).

Next Generation Sequencing

Genomic DNA Extraction and PCR amplification

The total community DNA was extracted using ProMega DNA kit (ProMega, USA). 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 (Kumbhare et al. 2015). The fungal Internal Transcribed Spacer (ITS) II region was targeted for taxonomic profiling amylolytic starters, *marcha* and *thiat*, which was subjected to amplification using ITS1 and ITS2 primers.

High-throughput Amplicon sequencing

The library preparation of ITS gene amplicons were done 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 (Caporaso et al. 2010). The sequences obtained from high throughput sequencing effort were submitted to National Centre for Biotechnology Information (NCBI) which is available under BioProject ID PRJNA376467. 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 (Masella et al. 2012). The assembled reads were subjected to quality filtering using via Quantitative Insights into Microbial Ecology (QIIME) 1.8 (Masella et al. 2012). Sequence reads were assigned 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% (Edgar et al. 2012). Taxonomic assignments were performed using RDP naïve bayesian classifier (Wang et al. 2007) Alpha diversity indices like Chao, Shannon and

Simpson were calculated via QIIME after rarefying all samples to the same sequencing depth Blaalid et al. (2013) and Bokulich et al. (2012).

PHYLOGENETIC ANALYSIS

The BLAST program was used for comparing DNA databases for sequence similarities available online on the EzTaxon server (http://eztaxon-e.ezbiocloud.net/). The phylogenetic tree was constructed by the Neighbor-joining method (Saitou and Nei 1987) using the CLUSTAL W program (Thompson et al. 1994). Molecular phylogenetic analysis was done by using the MEGA.7 software. The bootstrap consensus tree derived with 1000 replicates to Neighbor-joining method and Kimura 2-parameter. Numbers on branches depict the percent occurrence of a given branch during 1000 replicates.

BIOINFORMATICS ANALYSIS

The quality of raw ITS region from yeast isolates and PCR-DGGE band raw sequencing data were checked with the help of Sequence Scanner software (Applied Bio systems, USA) and the raw sequencing data alignment and analysis were done with the help of SEQMANN software (DNASTAR, USA), After the raw data alignment, BLAST program was used for comparing raw sequence databases for sequence similarities available on the NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1990; Zhao and Chu 2014). Construction of a phylogenetic tree done by the Neighbor-joining method (Saitou and Nei 1987) was performed using the CLUSTAL W program (Thompson et al. 1994). Shannon index of general diversity (H) and the richness of the microbial community as microbial diversity indices were done by following the method of Oguntoyinbo et al. (2011).

The raw sequences generated from MiSeq platform in high-throughput amplicon sequencing method was assembled using FLASH tool (Fast Length Adjustment of Short reads) 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 assigned to fungal operational taxonomic units (OTUs) by a closed reference-based OTU picking approach by using the UNITE reference databases. The OTU picking was carried out using the UCLUST method with similarity threshold of 97 % (Edgar 2010). Taxonomic assignments were done using RDP naïve bayesian classifier (Wang et al. 2007). Alpha diversity indices like Shannon, Shannon and Chao were calculated via QIIME piprline after rarefying all samples to the same sequencing depth Blaalid et al. (2013) and Bokulich et al. (2012).

NUCLEOTIDE ACCESSIONS

ITS-PCR

The raw sequences obtained from internal transcribed spacrer ITS region sequencing of isolated 46 yeast strains have been deposited in the NCBI GenBank under accession number: KY587119 - KY626335

PCR-DGGE

The raw sequences obtained from 202 bands of 26S rRNA gene of yeast from DGGE been deposited in the NCBI GenBank under accession number: KY594045 KY594246.

HIGH-THROUGHPUT SEQUENCING

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

DETERMINATION OF ENZYMATIC ACTIVITIES

Liquefying (a-amylase) activity

2 ml of 48-h old culture of yeasts was centrifuged at 17,000 rpm for 10 min, and the supernatant was filtered. The supernatant was diluted to a proper concentration for estimation of *a-amylase* and glucoamylase activities. The a-amylase activity of the particular yeast was determined by considerably modifying the method described by Tamang and Thapa (2006). Briefly, 0.1 ml of supernatant was incubated with 0.5 ml of soluble starch solution (1%, w/v), and 0.4 ml of phosphate buffer (0.1 M phosphate buffer for pH 7.0) and were incubated at 40°C for 10 min. The reaction was stoped by the addition of 1 ml of 3, 5-dinitrosalicylic acid and the generated reducing sugars were calculated in UV-VIS Spectrophotometer at OD 660 nm. One unit of a-amylase activity was defined as the amount of enzyme liberating 1 μmol of reducing sugars (glucose equivalents) per min at pH 7.0 at 40°C and was expressed as U/gm.

Glucoamylase Activity

Glucoamylase activity was estimated according to the modified method of Tamang and Thapa (2006). The reaction mixture containing 2 ml of 1% soluble starch solution (RM 089, HiMedia, Mumbai, India) in 2 ml of 100 mM acetate buffer (pH 5.0) and 0.5 ml of the supernatant (2 ml of 48-h old yeast culture was centrifuged at 17,000 rpm for 10 min, and the supernatant was filtered) was pre-incubated separately at 40°C for 5 min in a shaking water-bath. The 2 ml of 1% soluble starch solution was

added to the supernatant and incubated at 40°C for 10 min. After the 10 min of reaction, 1 ml of the reaction mixture was taken and glucose was determined by using UV-VIS Spectrophotometer at 660 nm absorbance of the resulting solution. One unit of glucoamylase activity was defined as the amount of enzyme, which releases 1 mg glucose in 1 min under the above condition. A unit of activity was expressed as mg glucose released per ml per 10 min and was expressed as U/gm.

ESTIMATION OF ALCOHOL

The ethanol yield of yeast isolates will be determined after growth at 28°C for 3, 4, 5 and 6 days in YM broth (HiMedia, M425) containing 10% glucose (Tsuyoshi et al. 2005). The Percent of ethanol produced by yeasts in YM broth were estimated by following the method of (Caputi et al. 1968) as well by spectrophotometric method (AOAC 2016). Then Ethanol standards were made by using ethanol-water solution in the range of 0–20 % ethanol (v/v). Potassium dichromate solution was prepared by adding 325 ml conc. H_2SO_4 to 400 ml distilled water in 1 litter volumetric flask. After mixing and cooling (8-9°C), 33.768 g $K_2Cr_2O_7$ was added and then final volume of 1 litter was made with distilled water at 20°C. Standard curve was prepared by taking 1 ml of each concentration of the standard solution [0-20% (v/v)] in a 100 ml volumetric flask containing 25 ml of potassium dichromate solution. The samples were heated at 60°C for 20 min in a water bath and then cooled and diluted to 50 ml with distilled water. Absorbance was recorded at a wavelength of 600 nm using UV Spectrophotometer (UV-visible spectrophotometer, Perkin Elmer). One ml of 72 hours old cultures broth was added directly to the distillation flask, diluted to 30 ml with distilled water and then distilled. Distillation was carried out at 60°C and 20 ml of distillate was collected in a 50 ml volumetric flask containing 25 ml of potassium

dichromate solution. The contents in the volumetric flask were heated at 60°C in a water bath for 20 min and final volume was made to 50 ml with distilled water. After mixing and cooling the contents of the flask, the absorbance was recorded at 600 nm. The amount of ethanol in each sample was determined by using the standard curve of ethanol. Alcohol produced in YM broth was estimated after 4, 5 and 6 days described above.

STATISTICAL ANALYSIS

Shannon index of general diversity (H) and the richness of the microbial community as microbial diversity indices were determined by following the method of Oguntoyinbo et al. (2011). Other graphical emphasis was done on *igraph* package in R Software and Graph Pad Software (Csardi and Nepusz, 2006). The significant difference in the alpha diversity indices of NGS data was performed by using the software QIIME. The results were obtained from enzymatic and ethanol production given as means value (±) standard deviation (Kim et al. 2014).

DOCUMENTATION OF TRADTIONAL METHODS OF AMYLOLYTIC STARERS OF NORTH EAST INDIA

All eight states of India located in North East regions 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. We documented starter culture-making technology practised by ethnic people of North East, which may reflect the traditional method of sub-culturing desirable inocula from previous batch to new culture using rice as base substrates by back-sloping method. Data collected were documented as below.

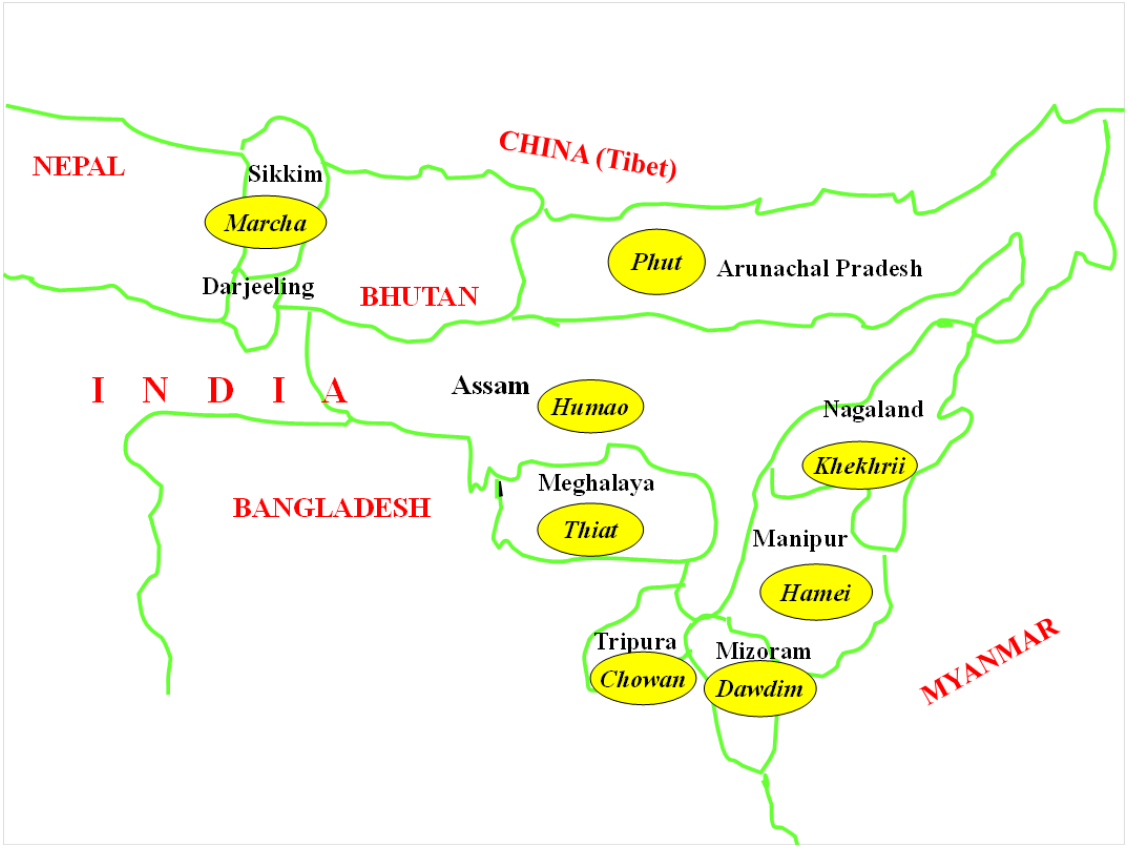


Figure 1. Map showing different collection sites of traditionally prepared ethnic amylolytic starters of North East India.

Table 1. Ethnic Amylolytic Starters of North East India

Starter	Substrates	Nature	Alcoholic products	States
Marcha	Rice, wild herbs, spices	Dry, mixed starter to ferment alcoholic beverages	*Kodo ko jaanr, Bhaati jaanr*	Sikkim
Humao	Rice-wheat, herbs	Dry, mixed starter	*Judima*	Assam
Hamei	Rice, wild herbs	Dry, mixed starter	*Aitaiba*	Manipur
Thiat	Rice-herbs	Starter to ferment alcoholic beverages	*Kiad*	Meghalaya
Chowan	Rice-herbs	Starter to ferment alcoholic beverages	*chuwak*	Tripura
Khekhrii	Germinated rice	Starter to ferment alcoholic beverages	*zutho/zhuchu*	Nagaland
Phut	Rice-herbs	Starter to ferment alcoholic beverages	*Apo*	Arun achal Pradesh
Dawdim	Rice-herbs	Starter to ferment alcoholic beverages	*Zawlaidi*	Mizoram

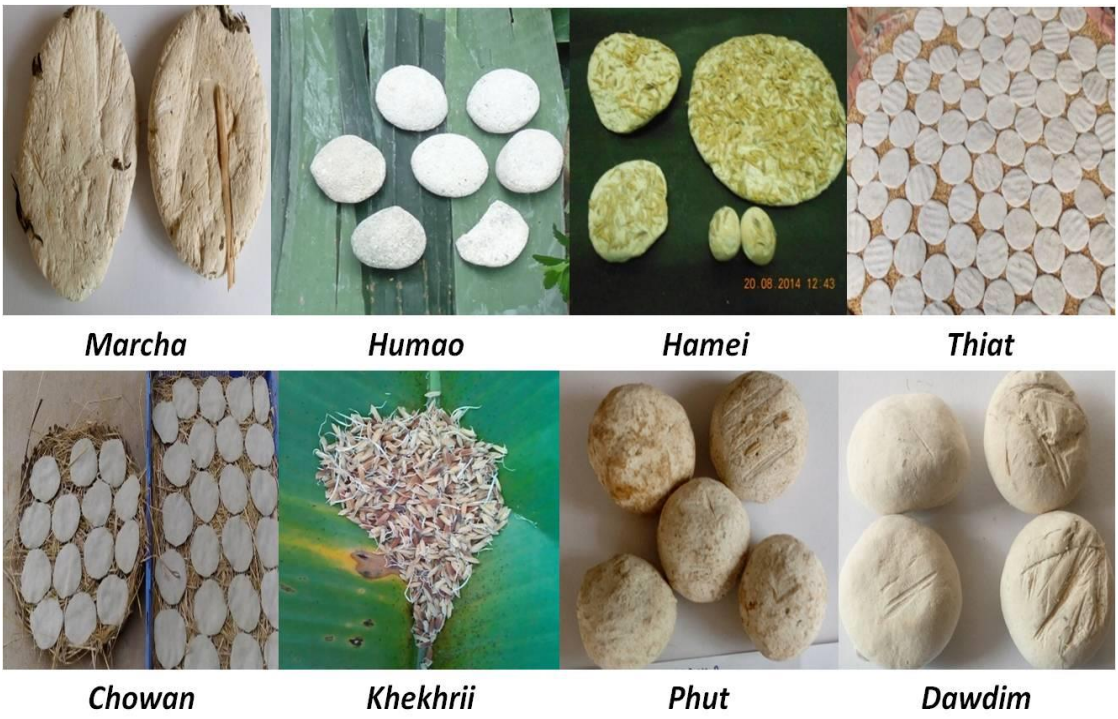


Figure 2. Amylolytic starter culture of North East India: *Marcha* of Sikkim, *Humao* of Assam, *Hamei* of Manipur, *Thiat* of Meghalaya, *Chowan* of Tripura, *Khekhri* of Nagaland, *Phut* of Arunachal Pradesh and *Dawidim* of Mizoram

MARCHA

Marcha is a dry, flat, creamy white and solid ball like starter of different size and shapeused to ferment starchy material into fermented beverages in Sikkim and Darjeeling hills in India, Bhutan and Nepal. The preparation processes of *marcha* in these regions are almost same.

Indigenous knowledge of preparation

During preparation of *marcha* firstly glutinous rice (*Oryza sativa)* is soaked in water for 8-10h (overnight) at room temperature. After soaking glutionous rice is crushed in a foot driven heavy weight wooden motar 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 glutionous rice. Then the powdered mixture of glutionos rice and wild parts of herbs are then mixed using water to make a thick paste or dough, from the mixed dough balls of different sizes are made. These newly prepared *marcha* balls are then dusted with the old powdered *marcha* which are used as source of an inoculum. The freshly prepared *marcha* cakes are then kept on the leaves of fern *Glaphylopteriolopsis erubeseens* (commonly known as *Pirey uneu*). After keeping the freshly prepared *marcha* cakes on the fresh leaves of fern it is further fully covered with the ferns and kept for incubation at room temperature for duration of 24 h. After 24 h of incubation the ferns are

removed and the *marcha* cakes are collected and dried naturaly in sunlight or kept at room temperature for about 5 days for sun drying (Figure. 3). The dried *marcha* cakes are then ready to sell in the local markets of Sikkim.

Figure 3. Preparation of *marcha in* Sikkim.

HUMAO

Huamo is a traditionally prepared rice-based ethnic amylolytic starter culture of Assam, commonly used for the preparation of *judima,* mild alcoholic beverage in rural parts of Assam.

Indigenous knowledge of preparation

Local glutinous rice (*Oryza sativa)* is soaked in water for about 3-4 h and mixed with various types of plants like leaves of Banana, Lwkwna, Dong-Phang-Rakhep (*Scoparia dulcis*) and Khantal leaves. These ingredients are taken in to the wooden mortar pestle (this set of apparatus is locally called Gaihen and ual) then ground together. The powdered rice is then sieved in a sandri (traditional sieve made of bamboo) to which little amount of water is added to mix it and make thick paste or dough. Then various sizes of small round to oval cakes areprepared from this dough which is then dusted with the old powdered amylolytic starter culture, used as a source of an inoculum. Once the fresh fresh *humao* cakes are are prepared it is kept and cover with paddy straw for 2-3 days for the incubation. Once the natural incubation completed the *humao* cakes are naturally sundried (Figure. 4). This process of natural drying continues for a couple of weeks until *humao* cakes becomes hard, and ready to be used for the preparation of alcoholic beveragesas well as to sell in the markets.

Figure 4. Preparation of *humao in* Assam

HAMEI

Hamei is an ethnic amylolytic mixed dry, round to flattened starter of Manipur. It is used for the preparation of rice-based alcoholic beverage called *aitanga* and distilled part *yu* in Manipur.

Indigenous knowledge of preparation

Hamei is prepared from local varieties of glutinous rice which is either soaked in water or used directly without soaking. The rice is mixed with the powdered bark of '*yangli*' (*Albizia myriophylla* Benth.) and a small amount of old powdered *hamei*. Then mixture is then powdered and dough is made by mixing a small amount of clean water to it. Then the round to flat balls are from the dough and kept for incubation over paddy husk in a bamboo basket, covered by sack clothes for 2-5 days at room temperature, and then it is drying naturally under sun for 2-4 days (Figure. 5). Once the fermentation completed it indicated by swelling of cakeand desired state aroma. These commercial amylolytic starters are prepared during summer (May–July) and dried *hamei* balls maintain their one year of shelf life.

Figure 5. Preparation of *hamei* in Manipur.

THIAT

Thiat is a dry tradtionally prepared amylolytic starter of Meghalaya which used for the preparation mild alcoholic beverage–*kiad.*

Indigenous knowledge of preparation

During the preparation of *thiat* firstly the glutinous rice washed, soaked and then powdered. The rice powder is now mixed with powdered, khaw-*iang-/hawiang* plants leaves with clean water and small pieces of cakes were made in size ranging from 4-5 cm in diameter and 0.8-1.0 cm in thickness and are kept for incubation in *malieng* and covered by *sla-pashor* after incubation the *thait* are exposed to sunlight for 4-5 days of drying after drying the cake becomes harden and it is now ready for storage and for prepertaion of local alcoholic beverage, *kiad* as wall well sells in the local markets of Meghalaya (Figure.6).

Glutinous Rice grain

↓

Soaked in water 15-20 minute

↓

Grinding in wooden mortar (*Thlong*) pestle (*Synrei*)

↓

Grinded rice is sieved and the powdered one is collected

← ***khaw-iang leaves* is usually cut into small pieces, dried and grinded.**

↓

Rice Powder and *khaw-iang* are mixed together with a little of water for making a paste

↓

Mixture is then kneaded (rolled) into a small dough-like mixture and it is sprinkled with the left-over powdered rice

↓

Incubation at an earthen oven for 2-3 days (winter) or over-night (summer)

↓

Thiat

Figure. 6 Preparation of *thiat* in Meghalaya.

CHOWAN

Chowan is an ethnic traditionally prepared amylolytic starter of Tripura used in preparation of local etnic mild alcoholic beverages.

Indigenous knowledge of preparation

During preparation of *chowan*, soaked glutinous rice is mixed with leaves and roots of various kinds of herbs and powdered previously prepared *chowan*. The above mixture is then made into paste by mixing the clean water and round to flat and oval cakes of varying sizes and shapes are made from the paste (Figure. 7). The freshly prepared *chowan* balls starters are naturally sun dried for 3-7 days and used for the preparation of local alcoholic beveagres and sell in to markets.

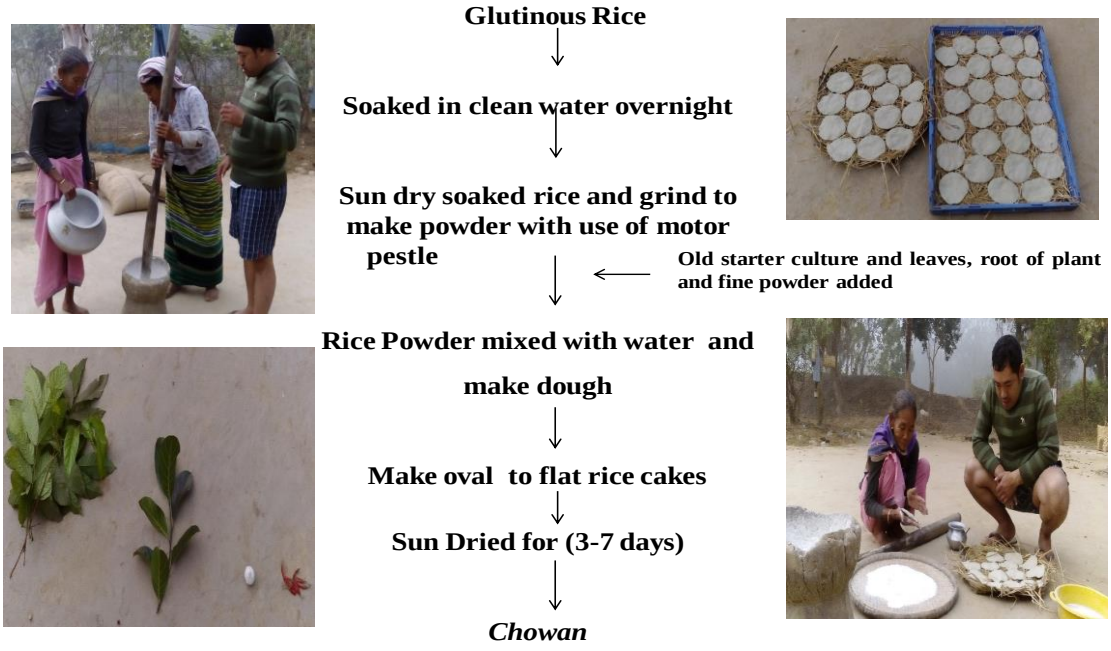


Figure 7. Preparation of *chowan* in Tripura

KHEKHRII

It a traditionally prepared ethnic amylolytic starter of Nagaland used to prepare local mild alcoholic beverage *zutho*. It is different from other ethnic amylolytic starter cultures of North-East India, since it is prepared by using unhulled glutinous rice grains.

Indigenous knowledge of preparation

Unhulled glutinous rice (dhan) is wash with water twice or thrice and then soaked into water for 2-5 days. Then it is kept and covered with *Khreihenyii leaves* and allowed for germination for 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 (Figure. 8).

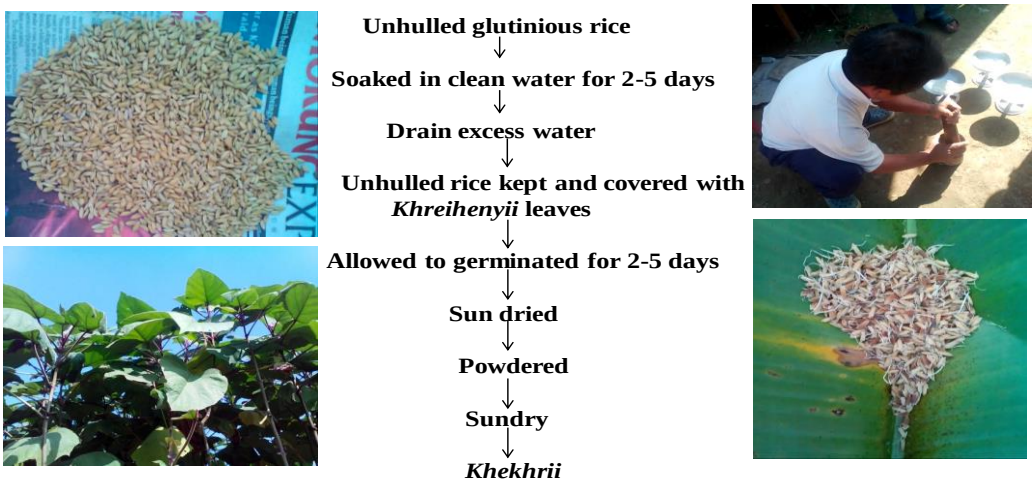


Figure. 8 Preparation of *khekhrii* in Nagaland.

PHUT

Phut is a round to flat white to dusty color traditionally prepared amylolytic starter by the Tagin community of Arunachal Pradesh. Apatani community and Nyshing community of Arunachal Pradesh also prepare this starter.

Indigenous knowledge of preparation

Preparation of *phut* includes local rice-flour, previously masde starter culture and leaves of wild herbs. The wild herbs used for preparation are locally known as "Nakail" (*Cinnamomum glanduliferum Mesissn*.) and *Ctuepatti* (*Cissampelos pareira Linn.* and *Khanoba* (*Clerodenderon viscosum Vent.*) respectively.The soaked glutinous rice is naturally dried in sun and powderwed using wooden mortar and pestle. Few leaves of the Nakali plant along with 5-7 old starter cakes fine powder are added to rice flour and mixed properly. The mixture is converted to paste by using water and make small round to flattened cakes (Figure. 9). Then they are covered in fresh leaves. Then this round to flate cakes are kept in to bamboo strips over the fire place for 1-2 days. There after they are naturally dried by sun drying for 5-10 days and store up to 6 months for the further use and sell.

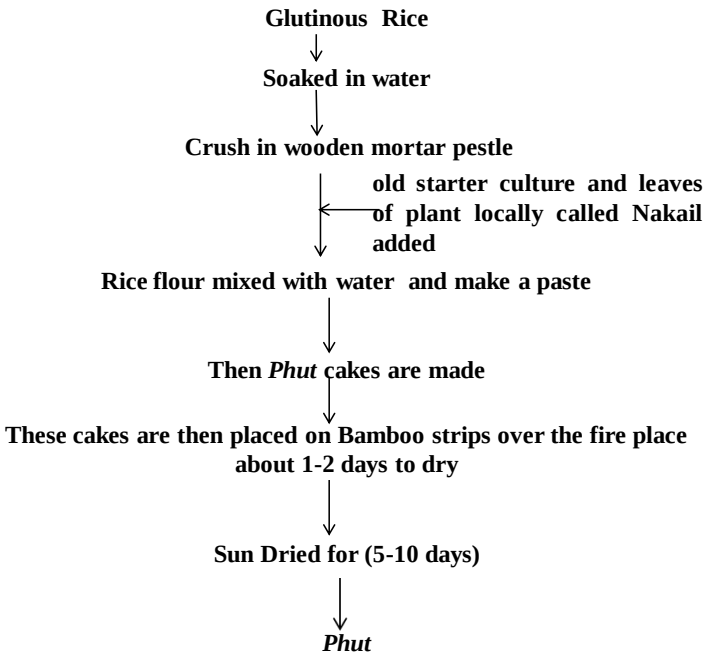


Figure 9. Preparation of *phut* in Arunachal Pradesh.

DAWDIM

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

Indigenous knowledge of preparation

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 (Figure 10).

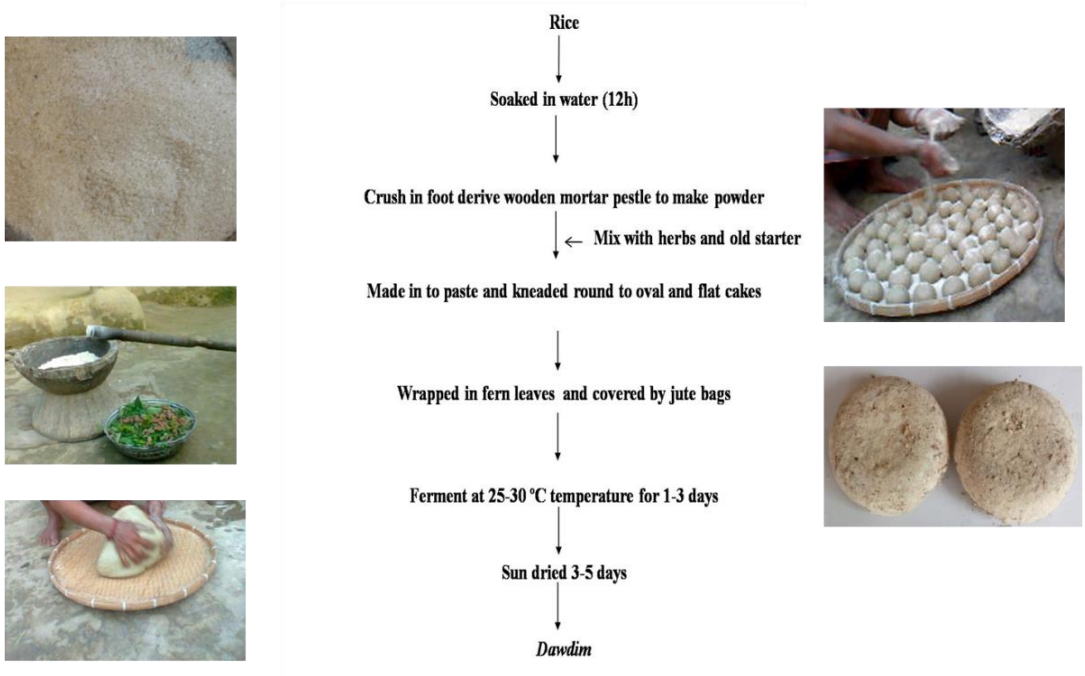


Figure 10. Preparation of *dawdim* in Mizoram

SOCIO-ECONOMIC IMPORTANCE

During our survey we observed that traditionally prepared starters are not only prepared at household level for personal use to prepare alcoholic beverages for drinking, but also at a commercial scale in some villages of North-East India, from where traditionally prepared starter cultures are supplied to the local markets. Some ethnic groups of people mostly rural women are economically dependent on the preparation of these amylolytic starter cultures We estimated an average price of traditionally prepared starter in local markets per piece is Re. 1 to Rs.10 per price, depending on size. 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. During our survey it was observed that 90% (ration to men) of rural women of North East practice the indigenous or native knowledge of preparation of starters right from cultivation of rice, post-harvest, preparation of starters and even selling at local markets for livelihood.

CULTURE DEPENDENT RESULTS

Phenotypic Charactetrization

The average load of yeast in all eight starters of North East India viz. *marcha, humao, hamei, thiat, phut, khekhrii, chowan* and *dawdim* (Table. 1) are 7.3 x 10^6cfu/g, 6.8 x 10^6cfu/g, 7.1 x 10^6cfu/g, 6.8 x 10^6cfu/g, 6.9 x 10^6cfu/g, 7.1 x 10^6cfu/g, 7.1 x 10^6cfu/g, 7.1 x 10^6cfu/g (Table. 2). A total of 386 yeasts strains were isolated from 40 samples of eight different amylolytic startersof North East India. Characterizations of yeasts were phenotypically done on the basis of colony morphology, cell morphology, sugar fermentation and sugar assimilation tests and the results were tabulated in (Table 3-43). All isolates fermented glucose, maltose, trehalose, sucrose, cellobiose, starch and galactose and all isolates assimilated arabinose, rhamnose, sucrose, xylose, cellobiose, starch and maltose Tentatively the following yeast genera were phenotypically identified as *Saccharomyces* (6.0%), *Pichia* (15.0%), *Candida* (14.0%), *Issatchankia* (15.0%), *Kluveromyces* (11.0%), *Schizosaccharomyces* (13.0%), *Saccharomycopsis* (8.0%) and *Torulopsis* (18.0%) were showed in (Figure. 14). On the basis of phenotypic results it was concluded that *Pichia* and *Candia* were dominant yeasts. All the tentatively yeasts genera such as *Saccharomyces* (5.4%), *Pichia*, *Candida Issatchankia*, *Kluveromyces*, *Schizosaccharomyces*, *Saccharomycopsis* and *Torulospora* showed the pseudo-mycelia (Figure. 26) except the *Candida* showed true-mycelia (Figure. 25). The ascospore structure of the phenotypically identified yeasts strains are *Saccharomyces* (Hat-shaped), *Pichia* (Hat-shaped), *Candida* (Oval shaped) (Figure. 30), *Issatchankia* (Spheroidal), *Kluveromyces* (Ellipsoidal)

Schizosaccharomyces (Globose), *Saccharomycopsis* (Hat-shaped) and *Torulopsis* (Spheroidal). All the strains of yeasts showed negative results for the nitrate reduction test except the *Pichia* strain (Table. 3). The pure yeasts colony were creamy white, cottony, soft to sticky, oval to circular in shapes (Figure. 13, 14).

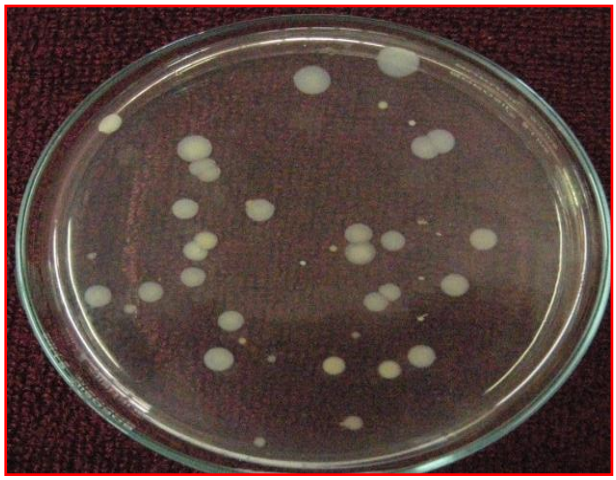


Figure 11. Isolation of dominant yeast from amylolytic starters by pour plate method on YMA

Figure 12. Isolation of pure yeast colony from amylolytic starters by streak plate method on YMA

Table 2. Average populations of yeasts in amylolytic starters of North East India

Samples	*Marcha*	*Humao*	*Hamei*	*Thiat*	*Phut*	*Khekhrii*	*Chowan*	*Dawdim*
States	Sikkim (n=10)	Assam (n=5)	Manipur (n=5)	Meghalaya (n=5)	Arunachal Pradesh (n=5)	Nagaland (n=5)	Tripura (n=5)	Mizoram (n=5)
cfu/g x 10^6	7.3 (7.2-7.4)	6.8 (6.5-7.1)	7.1 (7.0-7.2)	6.8 (6.7-7.0)	6.9 (6.8-7.1)	7.1 (7.0-7.2)	7.1 (7.0-7.2)	7.1 (7.0-7.3)

n = number of samples analysed; cfu, colony forming unit; ranges are given in parenthesis.

Table 3. Phenotypic characterization of yeast strains isolated from *marcha*

Isolate code		GM:Y1	GM:Y2	GM:Y3	GM:Y4	GM:Y5	GM:Y6	GM:Y7	GM:Y8	GM:Y9	GM:Y10
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 4. Phenotypic characterization of yeast strains isolated from *marcha*

Isolate code		GM:Y11	GM:Y12	GM:Y13	GM:Y14	GM:Y15	GM:Y16	GM:Y17	GM:Y18	GM:Y19	GM:Y20
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomyces***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 5. Phenotypic characterization of yeast strains isolated from *marcha*

Isolate code		GM:Y31	GM:Y32	GM:Y33	GM:Y34	GM: Y35	GM: Y36	GM: Y37	GM:Y38	GM:Y39	GM:Y40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+

Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Saccharomyces***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Kluyveromyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 6. Phenotypic characterization of yeast strains isolated from *marcha*

Isolate code		GM:Y31	GM:Y32	GM:Y33	GM:Y34	GM: Y35	GM: Y36	GM: Y37	GM:Y38	GM:Y39	GM:Y40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Saccharomyces***	***Sachharomycopsis***	***Issatchenkia***	***Pichia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Torulospora***	***Saccharomyces***

(+) = Positive reaction and (—) = Negative reaction

Table 7. Phenotypic characterization of yeast strains isolated from *marcha*

Isolate code		GM:Y41	GM:Y42	GM:Y43	GM:Y44	GM: Y45	GM: Y46	GM: Y47	GM:Y48	GM:Y49	GM:Y50
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+

	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Saccharomycopsis***	***Saccharomyces***	***Pichia***	***Kluyveromyces***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Saccharomycopsis***

(+) = Positive reaction and (—) = Negative reaction

Table 8. Phenotypic characterization of yeast strains isolated from *humao*

Isolate code		AS:Y1	AS: Y2	AS: Y3	AS:Y4	AS: Y5	AS: Y6	AS: Y7	AS: Y8	AS:Y9	AS:Y10
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 9. Phenotypic characterization of yeast strains isolated from *humao*

Isolate code		AS:Y11	AS:Y12	AS: Y13	AS:Y14	AS: Y15	AS: Y16	AS: Y17	AS:Y18	AS:Y19	AS:Y20
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+

	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomyces***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 10. Phenotypic characterization of yeast strains isolated from *humao*

Isolate code		AS:Y21	AS:Y22	AS:Y23	AS:Y24	AS: Y25	AS: Y26	AS:Y27	AS:Y28	AS:Y29	AS:Y30
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy, white	Creamy, white	Creamy smooth	Creamy, white	Creamy, white	Creamy, white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spherical	Spherical	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Saccharomyces***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Kluveromyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 11. Phenotypic characterization of yeast strains isolated from *humao*

Isolate code		AS :Y31	AS:Y32	AS: Y33	AS:Y34	AS: Y35	AS: Y36	AS: Y37	AS:Y38	AS:Y399	AS:Y40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Saccharomyces***	***Sachharomycopsis***	***Issatchenkia***	***Pichia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Torulospora***	***Saccharomyces***

(+) = Positive reaction and (—) = Negative reaction

Table 12. Phenotypic characterization of yeast strains isolated from *humao*

Isolate code		AS :Y41	AS:Y42	AS: Y43	AS:Y44	AS: Y45	AS: Y46	AS: Y47	AS:Y48	AS:Y49	AS:Y50
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Saccharomycopsis***	***Saccharomyces***	***Pichia***	***Kluyveromyces***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Saccharomycopsis***

Table 13. Phenotypic characteristics of yeasts strains isolated from *hamei*

Isolate code		HM:Y1	HM:Y2	HM:Y3	HM:Y4	HM:Y5	HM: Y6	HM: Y7	HM: Y8	HM:Y 9	HM:Y10
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (**—**) = Negative reaction

Table 14. Phenotypic characteristics of yeasts strains isolated from *hamei*

Isolate code		HM:Y11	HM:Y12	HM:Y13	HM:Y14	HM:Y15	HM:Y16	HM: Y17	HM:Y18	HM:Y1 9	HM:Y20
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	Oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Candida***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Sacharomyces***	***Pichia***	***Candida***	***Torulopsis***	***Pichia***	***Torulaspora***

(+) = Positive reaction and (**—**) = Negative reaction

Table 15. Phenotypic characteristics of yeasts strains isolated from *hamei*

Isolate code		HM:Y21	HM:Y22	HM:Y23	HM:Y24	HM:Y25	HM: Y26	HM: Y27	HM:Y28	HM:Y29	HM:Y30
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicleformation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	Bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Pichia***	***Trichosporon***	***Pichia***	***Issatchenkia***	***Sacharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Pichia***	***Torulaspora***

Table 16. Phenotypic characteristics of yeasts strains isolated from *hamei*

Isolate code		HM:Y31	HM:Y32	HM:Y33	HM:Y34	HM:Y35	HM: Y36	HM: Y37	HM: Y38	HM:Y 39	HM:Y40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Candida***	***Sacharomyces***	***Pichia***	***Issatchenkia***	***Sacharomyces***	***Pichia***	***Candida***	***Torulopsis***	***Pichia***	***Pichia***

Table 17. Phenotypic characteristics of yeasts strains isolated from *hamei*

Isolate code		HM:Y41	HM:Y42	HM:Y43	HM:Y44	HM:Y45	HM:Y46	HM: Y47	HM:Y48	HM:Y 49	HM:Y50
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Sacharomyces***	***Torulaspora***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 18. Phenotypic characterization of yeast strains isolated from *thiat*

Isolate code		ST :Y1	ST: Y2	ST: Y3	ST:Y4	ST: Y5	ST: Y6	ST: Y7	ST: Y8	ST:Y9	ST:Y10
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torichosporon***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 19. Phenotypic characterization of yeast strains isolated from *thiat*

Isolate code		ST :Y11	ST: Y12	ST: Y13	ST:Y14	ST: Y15	ST: Y16	ST: Y17	ST: Y18	ST:Y19	ST:Y20
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 20. Phenotypic characterization of yeast strains isolated from *thiat*

Isolate code		ST:Y21	ST:Y22	ST:Y23	ST:Y24	ST: Y25	ST: Y26	ST:Y27	ST:Y28	ST:Y29	ST:Y30
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy, white	Creamy, white	Creamy smooth	Creamy, white	Creamy, white	Creamy, white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spherical	Spherical	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+

	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Candida***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Sacharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Pichia***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 21. Phenotypic characterization of yeast strains isolated from *thiat*

Isolate code		ST:Y31	ST:Y32	ST:Y33	ST:Y34	ST: Y35	ST: Y36	ST: Y37	ST:Y38	ST:Y39	ST:Y40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Pichia***	***Trichosporon***	***Pichia***	***Issatchenkia***	***Sacharomyces***	***Kluyveromyces***	***Candida***	***Trichosporon***	***Pichia***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 22. Phenotypic characterization of yeast strains isolated from *thiat*

Isolate code		ST:Y41	ST:Y42	ST:Y43	ST:Y44	ST: Y45	ST: Y46	ST: Y47	ST:Y48	ST:Y49	ST:Y50
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Candida***	***Pichia***	***Pichia***	***Issatchenkia***	***Sacharomyces***	***Sacharomyces***	***Candida***	***Torulaspora***	***Pichia***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 23. Phenotypic characterization of yeast strains isolated from *phut*

Isolate code		AP :Y1	AP: Y2	AP: Y3	AP:Y4	AP: Y5	AP: Y6	AP: Y7	AP: Y8	AP:Y9	AP:Y10
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Saccharomyces***	***Saccharomycopsis***	***Issatchenkia***	***Pichia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Saccharomyces***

(+) = Positive reaction and (—) = Negative reaction

Table 24. Phenotypic characterization of yeast strains isolated from *phut*

Isolate code		AP :Y11	AP:Y12	AP: Y13	AP:Y14	AP: Y15	AP: Y16	AP: Y17	AP:Y18	ST:Y19	ST:Y20
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+129	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Pichia***	***Kluveromyces***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Schizosaccharomyces***	***Saccharomyces***

(+) = Positive reaction and (—) = Negative reaction

Table 25. Phenotypic characterization of yeast strains isolated from *phut*

Isolate code		AP:Y21	AP:Y22	AP:Y23	AP:Y24	AP: Y25	AP: Y26	AP:Y27	AP:Y28	AP:Y29	AP:Y30
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy, white	Creamy, white	Creamy smooth	Creamy, white	Creamy, white	Creamy, white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spherical	Spherical	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Kluyveromyces***	***Candida***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Saccharomyces***

(+) = Positive reaction and (—) = Negative reaction

Table 26. Phenotypic characterization of yeast strains isolated from *phut*

Isolate code		AP:Y31	AP:Y32	AP:Y33	AP:Y34	AP: Y35	AP: Y36	AP: Y37	AP:Y38	AP:Y39	AP:Y40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Pichia***	***Trichosporon***	***Pichia***	***Issatchenkia***	***Sacharomyces***	***Kluyveromyces***	***Candida***	***Torulospora***	***Pichia***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 27. Phenotypic characterization of yeast strains isolated from *phut*

Isolate code		AP:Y41	AP:Y42	AP:Y43	AP:Y44	AP: Y45	AP: Y46	AP: Y47	AP:Y48	AP:Y49	AP:Y50
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Pichia***	***Trichosporon***	***Saccharomyces***	***Issatchenkia***	***Saccharomyces***	***Kluyveromyces***	***Candida***	***Torulaspora***	***Pichia***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 28. Phenotypic characterization of yeast strains isolated from *khekhrii*

Isolate code		KY:1	KY:2	KY:3	KY: 4	KY: 5	KY:6	KY:7	KY: 8	KY:9	KY: 10
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Candida***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 29. Phenotypic characterization of yeast strains isolated from *khekhrii*

Isolate code		KY:11	KY:12	KY:13	KY:14	KY:15	KY:16	KY:17	KY: 18	KY:19	KY: 20
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 30. Phenotypic characterization of yeast strains isolated from *khekhrii*

Isolate code		K:Y21	KY:22	KY:23	KY:24	KY: 25	KY: 26	KY:27	KY: 28	KY: 29	KY: 30
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy, white	Creamy, white	Creamy smooth	Creamy, white	Creamy, white	Creamy, white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spherical	Spherical	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Pchia***	***Candia***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

Table 31. Phenotypic characterization of yeast strains isolated from *khekhrii*

Isolate code		KY 31	KY: 32	KY: 33	KY: 34	KY : 35	KY: 36	KY: 37	KY: 38	KY: 39	KY: 40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Pichia***	***Candida***	***Kluveromyces***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

Table 32. Phenotypic characterization of yeast strains isolated from *khekhrii*

Isolate code		KY:41	KY:42	KY:43	KY:4 4	KY:45	KY:46	KY:47	KY: 48	KY:49	KY: 50
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+

	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Issatchenkia***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Candida***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Pichia***

(+) = Positive reaction and (—) = Negative reaction

Table 33. Phenotypic characteristics of yeasts strains isolated from *dawdim*

Isolate code		MY:1	MY:2	MY:3	MY: 4	MY: 5	MY:6	MY:7	MY: 8	MY:9	MY: 10
Colony morphology		Cream y white	Creamy, white	Cream y, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circula r	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+

	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Candida***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***KY: 8 Torulopsis***	***Schizosaccharomyces***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 34. Phenotypic characteristics of yeasts strains isolated from *dawdim*

Isolate code		MY:11	MY:12	MY:13	MY:14	MY:15	MY:16	MY:17	MY: 18	MY:19	MY: 20
Colony morphology		Creamy white	Creamy, white	Creamy , smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicleformation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 35. Phenotypic characteristics of yeasts strains isolated from *dawdim*

Isolate code		MY:21	KY:22	MY:23	MY:24	MY: 25	MY: 26	MY:27	MY: 28	MY: 29	MY: 30
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Pchia***	***Candia***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 36. Phenotypic characteristics of yeasts strains isolated from *dawdim*

Isolate code		MY 31	MY: 32	MY: 33	MY: 34	MY : 35	MY: 36	MY: 37	MY: 38	MY: 39	MY: 40
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Pichia***	***Candida***	***Kluveromyces***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 37. Phenotypic characterization of yeast strains isolated from *chowan*

Isolate code		MY:41	MY:42	MY:43	MY:4 4	MY:45	MY:46	MY:47	MY: 48	MY:49	MY: 50
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicleformation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+

	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
	Tentative identification	***Issatchenkia***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Candida***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Pichia***

(+) = Positive reaction and (—) = Negative reaction

Table 38. Phenotypic characterization of yeast strains isolated from *chowan*

Isolate code		CH:Y1	CH:Y2	CH:Y3	CH:Y:4	CH:Y 5	CH:Y6	CH:Y7	CH:Y8	CH:Y9	CH:Y10
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–

	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+
	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Candida***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Torulaspora***

(+) = Positive reaction and (—) = Negative reaction

Table 39. Phenotypic characterization of yeast strains isolated from *chowan*

Isolate code		CH:Y11	CH:Y12	CH:Y13	CH:Y14	CH:Y15	CH:Y16	CH:Y17	CH:Y18	CH:Y19	CH:Y20
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white	Creamy smooth	Creamy white	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		—	—	—	+	+	—	—	—	—	—
Growth at 40ºC		+	+	+	—	—	—	—	—	+	+
Growth at 45ºC		+	—	—	—	—	—	—	+	—	—
Pellicle formation		+	+	+	+	—	+	—	—	—	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		—	—	+	—	—	—	—	—	—	—
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	—	+	+	+	+	+	+
	Sucrose	+	+	+	—	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	—	—	—	—	—	+	—	—	—	—
	Xylose	+	+	—	—	—	—	—	—	—	—
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	—	+	—	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 40. Phenotypic characterization of yeast strains isolated from *chowan*

Isolate code		CH:Y21	CH:Y22	CH:Y23	CH:Y24	CH:Y25	CH:Y26	CH:Y27	CH:Y28	MH:Y29	MH:Y30
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy, white	Creamy, white	Creamy smooth	Creamy, white	Creamy, white	Creamy, white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval	Oval to circular	Oval	oval	Circular to oval
Growth at 37ºC		–	–	–	+	+	–	–	–	–	–
Growth at 40ºC		+	+	+	–	–	–	–	–	+	+
Growth at 45ºC		+	–	–	–	–	–	–	+	–	–
Pellicle formation		+	+	+	+	–	+	–	–	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Multilateral
Ascospore		Ellipsoidal	Spherical	Spherical	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–	–	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+	+	+	+	+
	Sucrose	+	+	+	–	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+	–	–	–	–
	Xylose	+	+	–	–	–	–	–	–	–	–
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+	–	+	+	+

	Cellobiose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+	+	+	+	+
	Raffinose	+	+	+	+	+	+	—	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Starch	+	+	+	+	+	—	+	+	+	+
	Rhamnose	+	+	+	+	—	+	+	+	—	+
Tentative Identification		***Torulaspora***	***Saccharomycopsis***	***Pichia***	***Issatchenkia***	***Pchia***	***Candia***	***Candida***	***Torulopsis***	***Schizosaccharomyces***	***Issatchenkia***

(+) = Positive reaction and (—) = Negative reaction

Table 41. Phenotypic characterization of yeast strains isolated from *marcha*

Isolate code		CHY 31	CHY:32	CHY:33	CHY:34	CHY :35	CHY:36
Colony morphology		Creamy white	Creamy, white	Creamy, smooth	Creamy, smooth	Creamy white	Creamy white
Cell morphology		Oval to circular	Oval to spheroid	Oval	Oval	Oval to circular	Oval
Growth at 37ºC		–	–	–	+	+	–
Growth at 40ºC		+	+	+	–	–	–
Growth at 45ºC		+	–	–	–	–	–
Pellicle formation		+	+	+	+	–	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo
Budding type		Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral
Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal
Nitrate reduction		–	–	+	–	–	–
Sugar Assimilation	Galactose	+	+	+	+	+	+
	Maltose	+	+	+	–	+	+
	Sucrose	+	+	+	–	+	+
	Glucose	+	+	+	+	+	+
	Lactose	–	–	–	–	–	+
	Xylose	+	+	–	–	–	–
	Arabinose	+	+	+	+	+	+
	Trehalose	+	+	+	+	–	+

	Cellobiose	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+
Sugar Fermentation	Galactose	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+
	Trehalose	—	—	—	+	+	+
	Raffinose	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+
	Starch	+	+	+	+	+	—
	Rhamnose	+	+	+	+	—	+
Tentative Identification		***Pichia***	***Candida***	***Kluveromyces***	***Issatchenkia***	***Schizosaccharomyces***	***Kluyveromyces***

(+) = Positive reaction and (—) = Negative reaction

Tables 42. Grouping of total isolates of yeasts from all 40 samples of amylolytic starters of North East India on the basis of fermentation, and assimilation of sugars and other phenotypic tests.

Parameters	**Tentative identity**							
	Saccharomyces	*Pichia*	*Candida*	*Issatchankia*	*Kluveromyces*	*Schizosaccharomyces*	*Saccharomycopsis*	*Torulopsis*
Total isolates:	26	58	53	58	42	52	29	68
Sugar fermented:								
Lactose	+ (3), − (40)	−	+ (6), −(50)	−	−	−	−	−
Raffinose	+ (37), −(6)	+(56), −(4)	+ (57), −(3)	+	+	+	+ (18), − (5)	+ (56), −(4)
Xylose	+ (39), − (4)	+ (55), −(5)	+ (54), −(6)	+ (45), −(5)	+	+ (50), −(2)	+ (20), − (3)	+ (55), −(5)
Sugar assimiated:								
Trehalose	+ (40), −(3)	+(50), −(10)	+ (55), − (5)	+ (46), −(5)	+	+ (50), −(2)	+ (20), −(3)	+ (50), −(10)
Lactose	+ (3), − (40)	+	+ (4), −(52)	−	+	+ (4), −(48)	+ (3), − (20)	−

Raffinose	+ (39), −(4)	+ (51), − (5)	+ (50), −(6)	+ (47), −(4)	+ (38), −(3)	+ (47), −(5)	+ (20), −(3)	+ (56), −(4)
Melibiose	+ (40), −(3)	+ (55), −(5)	+ (54), −(6)	+	+	+	+	+
True/pseudo-mycelia	Pseudo-mycelia	Pseudo-mycelia	True mycelia	Pseudo-mycelia	Pseudo-mycelia	Pseudo-mycelia	Pseudo-mycelia	Pseudo-mycelia
Ascospore	Hat-shaped	Hat-shaped	Oval shaped	Spheroidal	Ellipsoidal	Globose	Hat-shaped	Spheroidal
Representative strains	GM:Y12, AS:Y12, HM:Y15, ST:Y46, AP:Y45, M:Y1, CH:Y22	GM:Y34, AS:Y3, HM:Y3, ST:Y3, AP:Y4, KY:Y3, M:Y49, CHY:34	GM:Y37, AS:Y7, HM:Y7 ST:Y41, AP:Y22, KY:45, MY:47, CHY:37	GM:Y4, AS:Y4, HM:Y50, ST:Y24, AP:Y3,KY:Y4, M:Y3, CHY:36,	GM:Y29, AS:Y6, HM:Y26, ST:Y36, AP:Y6, KY:33, M:Y6, CM:Y10,	AS:Y45, HM:Y9, ST:Y49, AP:Y15, KY:Y5, M:Y9, CH:Y15	GM:Y22, AS:Y2, HMY12, ST:Y12, AP:Y2, KY:Y42, M:Y2, CH:Y22	GM:Y1, AS:Y1, HM:Y28, ST:Y30, AP:Y38, KY:Y10, M:Y38, CM:Y18
All isolates fermented glucose, maltose, trehalose, sucrose, cellobiose, starch and galactose All isolates assimilated arabinose, rhamnose, sucrose, xylose, cellobiose, starch and maltose								

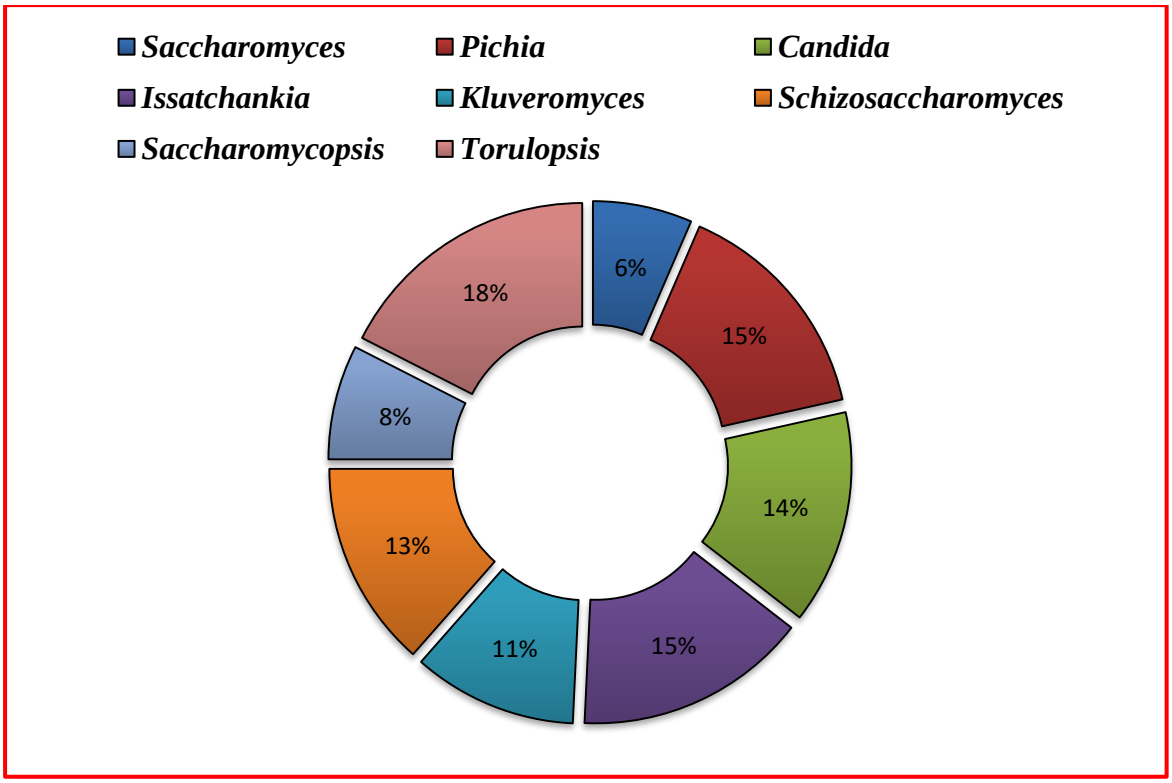


Figure 13.Distribution of yeasts in all amylolytic starters identified by phenotypic tests.

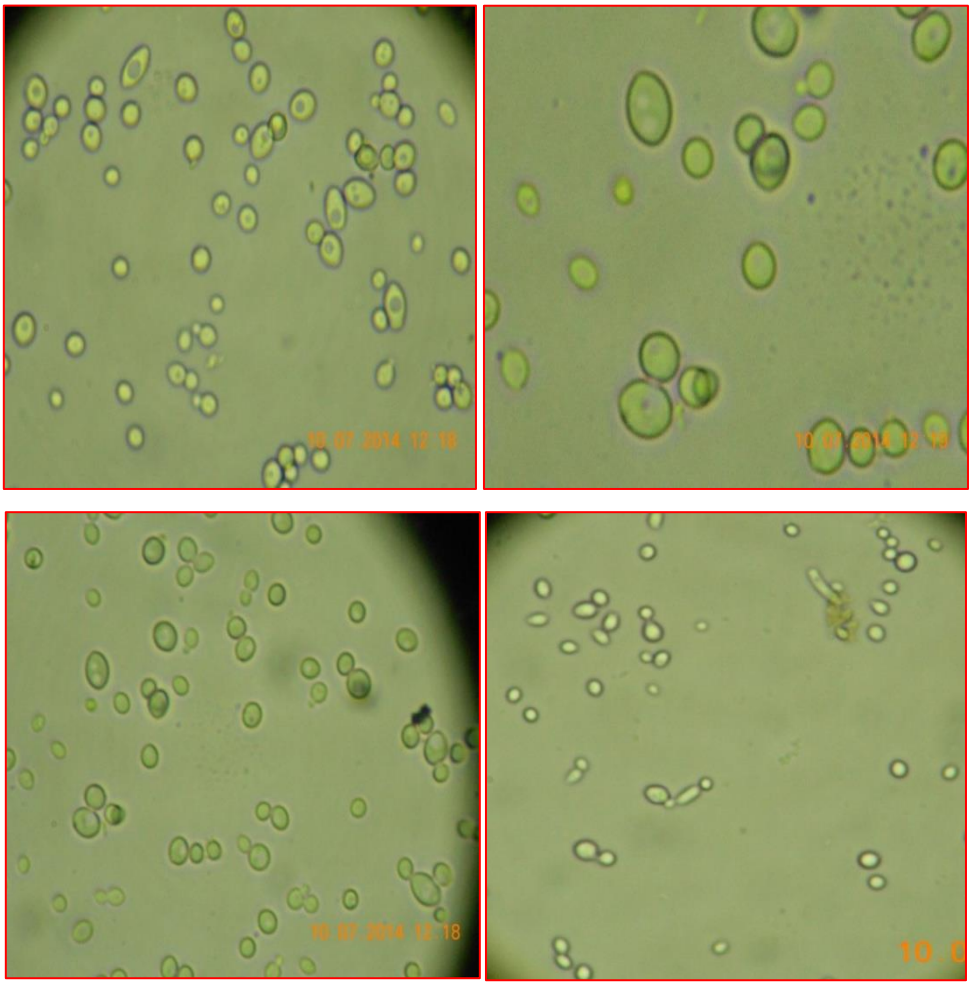


Figure14. Budding pattern of *Saccharomycopsis fibuligera* (STY21) and *Saccharomyces cerevisiae* (CHY22) under 100X Phase Contrast Microscope

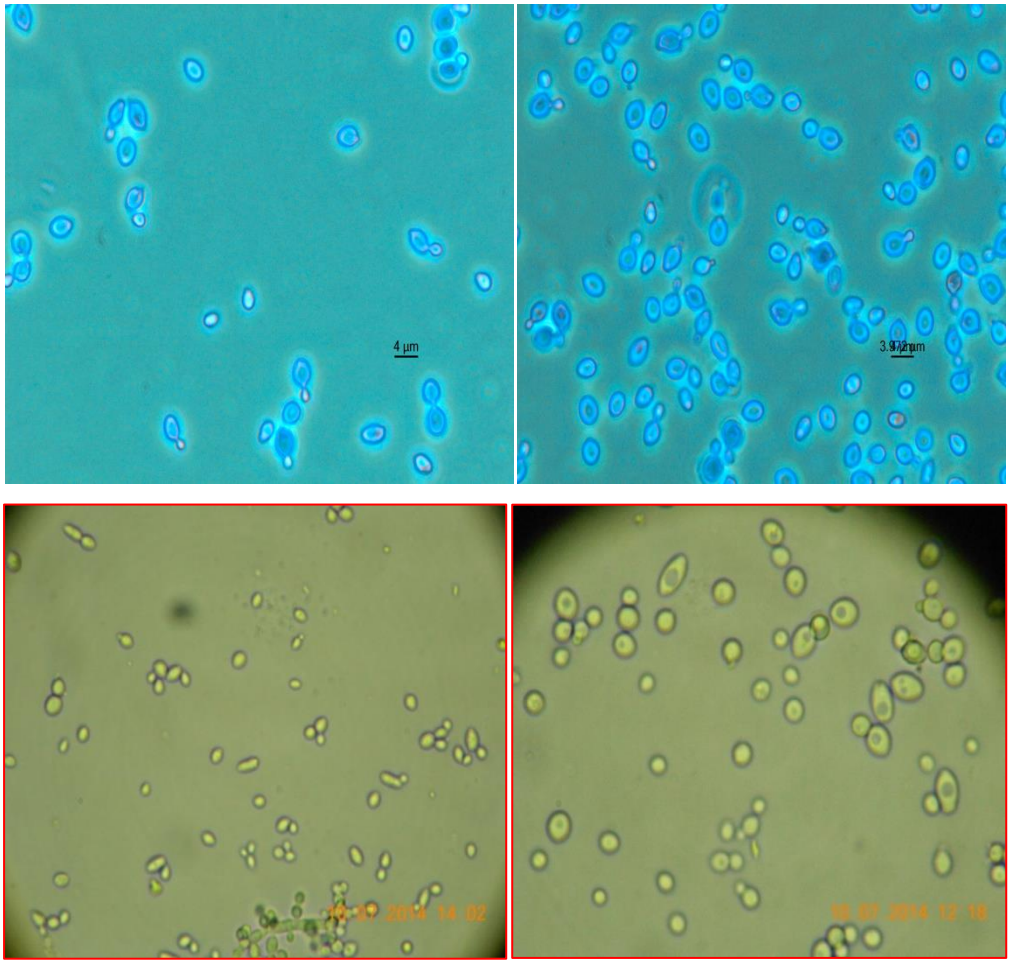


Figure 15. Budding pattern of *Pichia anomala* (GMY12) and *Candida glabrata* (CHY28) under 100X Phase Contrast Microscope

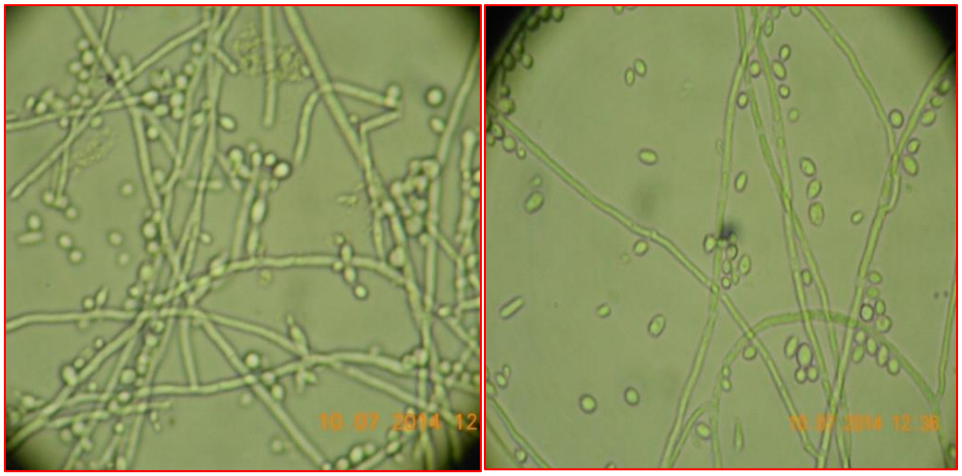


Figure 16. Microscopic view true mycelium of *Pichia kudriavzevii* (HY7) *Candida glabrata* (CHY28) under 100X Phase Contrast Microscope

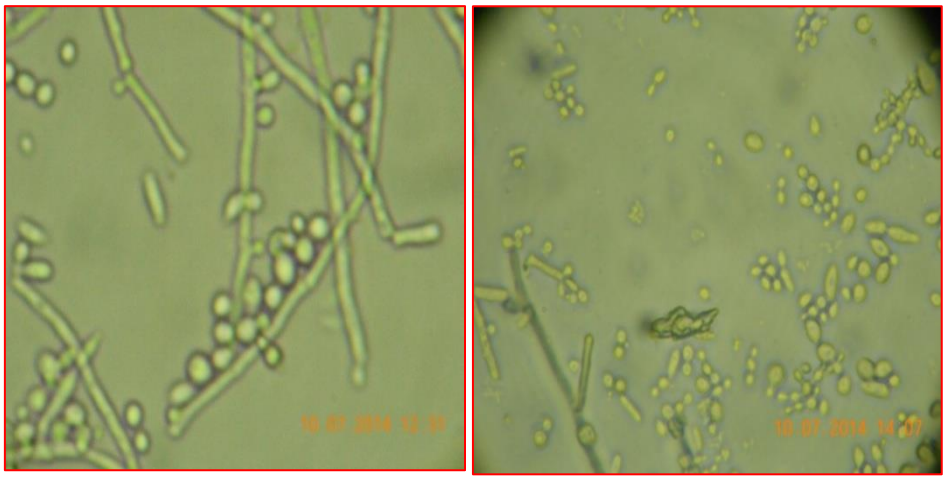


Figure 17. Microscopic view of simple pseudo mycelium of *Saccharomycopsis fibuligera* (XTY15) and *wickerhamomyces anomalus* (KY38) under 100X Phase Contrast Microscope.

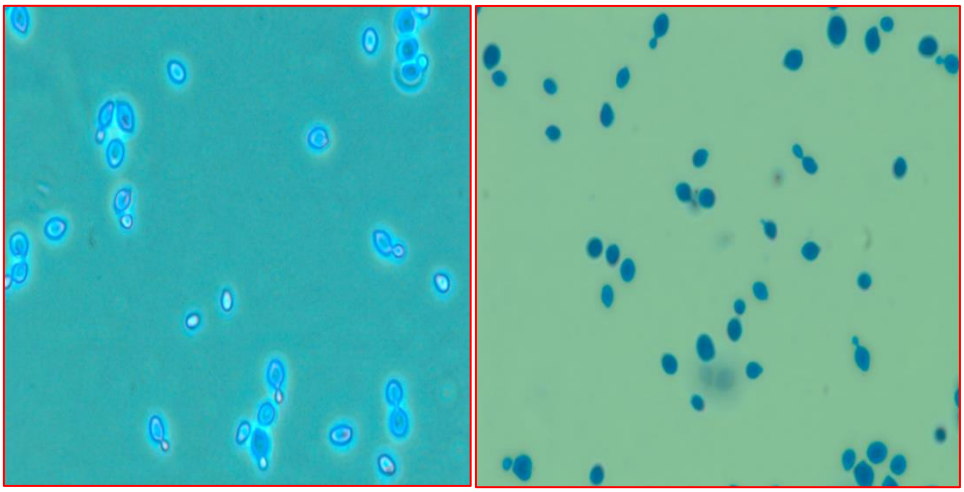


Figure 18. Microscopic view of *Saccharomycopsis fibuligera* (STY15) *Saccharomyces cerevisiae* (CHY22) under 100X Phase Contrast Microscope.

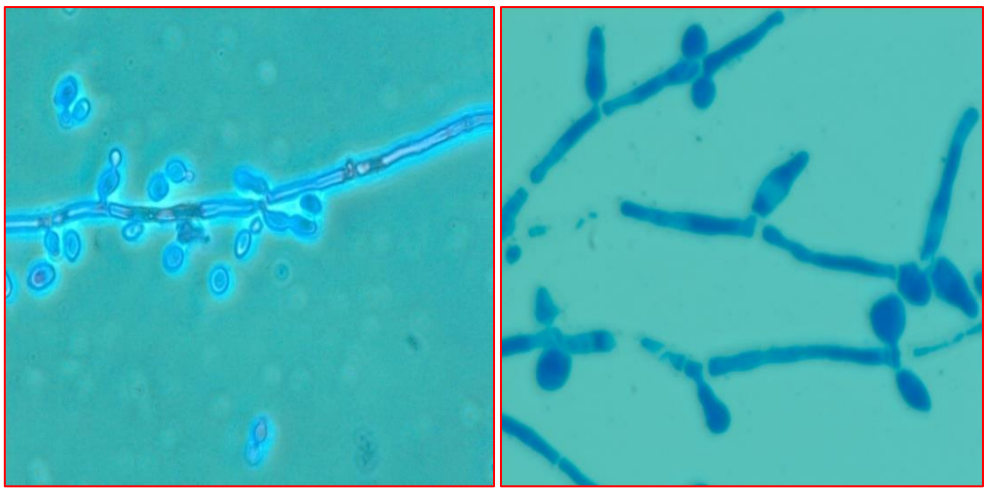


Figure 19. Microscopic view of *Candida glabrata* (MY30) and *Pichia anomala* (KY27) under 100X Phase Contrast Microscope.

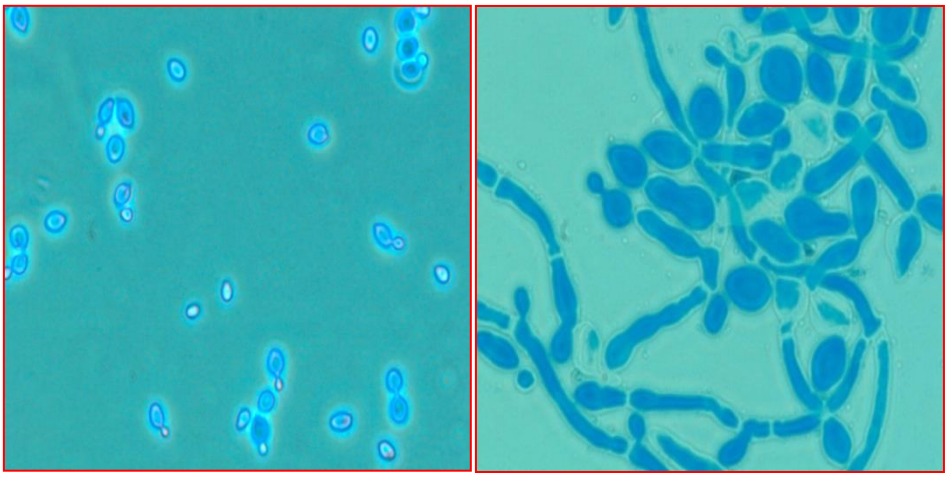


Figure 20. Microscopic view of *Wickerhamomyces anomalus* (MY57) *and Pichia terricola* (STY24) under 100X Phase Contrast Microscope.

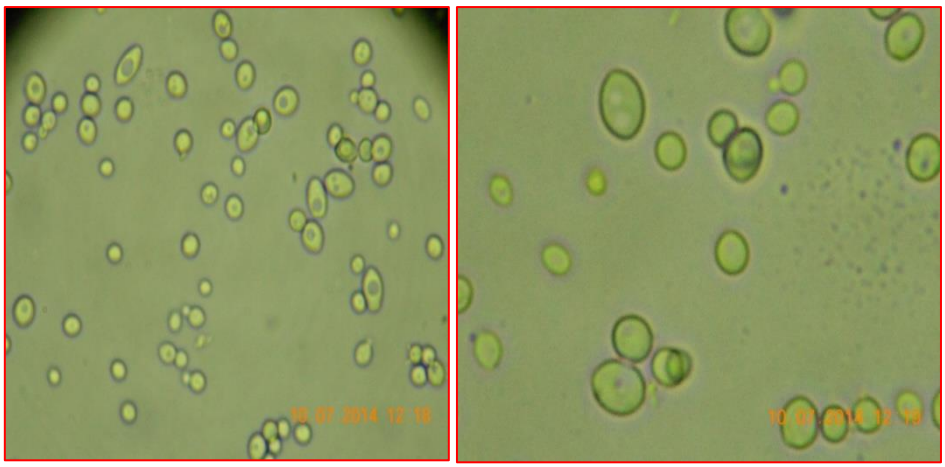


Figure 21. Microscopic view of ascospores of *Wickerhamomyces anomalus* (CHY39) and *Saccharomycopsis fibuligera* (STY21) under 100X microscope Phase Contrast Microscope.

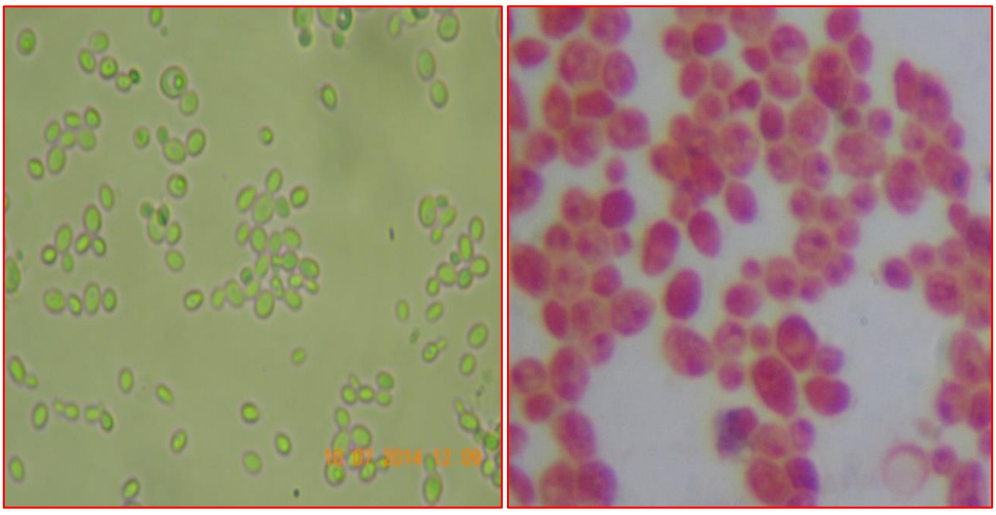


Figure 22. Microscopic view of ascospores of *Saccharomyces cerevisiae* (CHY22) and *Candida glabrata* (AH45) under 100X Phase Contrast Microscope.

BIOLOG IDENTIFICATION TEST

The Biolog identification system was used for the identification of 60 representative yeasts out of 386 isolates from ethnic amylolytic starters of North East India. The yeasts strains are *Pichia anomalus* (CHY38, CHY39, MY15, MY20, MY20, MY47, MY3, STY15, MY, MY3, MY8, HSY7, HY7, ASY3, ASY5, ASY4, KY8, KY18, GMY5, GM29, GMY29, STY20, STY21, STY24, STY6, STY12, STY3); *Saccharomycopsis fibuligera* (STY15,MY7); *Candida glabrata* (STY6, STY21, STY15, HY7, MY57, CHY28) *Debromyces castelii* (CHY28, STY3, STY12); *Pichia sydowiorum* (CHY39, XTY20), *Phichia onychis* (CX44, MY30); *Debromyces polymorphus* (CHX26, STY49), *Issatchenkia orientalis* (CHX39); *Phaphia rhodozymas* (GMY46); *Rhodotorula aurantaea* (MY9); *Endomyces fibuligera* (KY45, GMY29)*; Rhodotolura bacarum* (GMY12)*; Zygosaccharomyces bailii* (GMY1)*; Pichia trelalophila* (KY38), *Rhhodotorula acheniorium* (KY20), *Pichia subpeliculum* (KY27), *Pichia trelalophila* (KY38), *Pichia guillermondii* (MY6), and *Saccharomyces ceriviciae* (CHY22), were identified by using the metabolic fingerprints generated by Biolog identification system using biolg plate wells and incubated at 37 °C for 24-72 h, when positive results were recorded according to colour changes (Table 43) Their metabolic capacities were also assessed by comparing with the yeast database metabolic fingerprint the result revealed that maximum identified yeast species were associated with amylolytic starter having ≥0.75% probability and ≥0.7 similarities index value. We were successfully identified to 60 cultures wherein very few Biolog identified yeasts strains were showing ≥0.70% probability and ≥0.5 similarities index value (Table 44). The yeasts

strain *Pichia terricola* showed highest ≥0.974% probability with ≥0.77 similarities index value. It is observed that the results from Biolog were revealing more diversity of yeasts than phenotypic characterization and it presented in (Figure. 44). The results obtained were automatically read and analysed using MicroLog 3 software, which provided the most probable genera and species of the tested culture. The Biolog results showed the dominance of following yeasts viz., *Candia glabrata* (12%), *Pichia anomalus* (52%), *Debromyces castelii* (4%), *Debromyces polymorphus* (2%), *Phichia onychis* (2%), *Endomyces fibuligera* (4%), *Phaphia rhodozymas* (2%), *Pichia subpeliculum* (2%), *Pichia terricola* (4%), *Pichia trelalophila* (2%), *Rhhodotorula acheniorium* (2%), *Rhodotolura bacarum* (2%), *Rhodotorula aurantaea* (2%), *Saccharomyces ceriviciae* (2%), *Saccharomycopsis fibuligera* (4%), *Zygosaccharomyces bailii* (2%) in all amylolytic starters (Figure.23). The phylum level distribution of yeast in amylolytic starter analysed by biolog technique were Ascomycota, Basidiomycota, Zygomycota, Mucoromycotina, and Dothideomycetes (Figure. 40). Among the culture dependent-techniqiues Biolog results showed maximum diversity in all the eight amylolytic starters of North East India.

Table 43. Biolog identification of yeast stains isolated from different amylolytic starters of North East India

Sl. No	isolate code	Identified strains	Samples
1	CHY28	*Debromyces castelii*	*chowan*
2	CHY39	*Pichia sydowiorum*	*chowan*
3	CX44	*Phichia onychis*	*chowan*
4	CHX26	*Debromyces polymorphus*	*chowan*
5	CHX39	*Issatchenkia orientalis*	*chowan*
6	CHY22	*Saccharomyces ceriviciae*	*chowan*
7	CHY38	*Pichia anomalus*	*chowan*
8	CHY28	*Candida glabrata*	*chowan*
9	CHY39	*Pichia anomalus*	*chowan*
10	MY15	*Pichia anomalus*	*dawdim*
11	MY9	*Rhodotorula aurantaea*	*dawdim*
12	MY20	*Pichia anomalus*	*dawdim*
13	MY30	*Pichia onychis*	*dawdim*
14	MY47	*Pichia anomalus*	*dawdim*
15	MY57	*Candia glabrata*	*dawdim*
16	MY3	*Pichia anomalus*	*dawdim*
17	MY6	*Pichia guillermondii*	*dawdim*
18	STY15	*Pichia anomalus*	*dawdim*
19	MY5	*Pichia anomalus*	*dawdim*
20	MY3	*Pichia anomalus*	*dawidim*
21	MY6	*Pichhia anomalus*	*dawidim*
22	MY8	*Pichia anomalus*	*dawidim*
23	MY8	*Saccharomycopsis fibuligera*	*dwadim*
24	HSY7	*Pichia anomalus*	*hamei*
25	AH45	*Pichia anomalus*	*hamei*
26	HSY7	*Candida glabrata*	*hamei*
27	ASY3	*Pichia anomalus*	*humao*
28	ASY5	*Pichia anomalus*	*humao*
29	ASY5	*Pichia terricola*	*humao*
30	ASY4	*Pichia anomalus*	*humao*
31	KY8	*Pichia anomalus*	*khekhriii*
32	KY20	*Rhhodotorula acheniorium*	*khekhriii*
33	KY18	*Pichia anomalus*	*khekhriii*
34	KY27	*Pichia subpeliculum*	*khekhriii*
35	KY38	*Pichia trelalophila*	*khekhriii*
36	KY45	*Endomyces fibuligera*	*khekhriii*
37	GMY1	*Zygosaccharomyces bailii*	*marcha*
38	GMY5	*Pichia anomalus*	*marcha*
39	GMY12	*Rhodotolura bacarum*	*marcha*
40	GMY29	*Endomyces fibuligera*	*marcha*
41	GMY46	*Phaphia rhodozymas*	*marcha*
42	GM29	*Pichia anomalus*	*marcha*

43	GM29	*Pichia anomalus*	*marcha*
44	XTY20	*Pichia sydowiorum*	*phut*
45	STY15	*Candida glabrata*	*phut*
46	STY20	*Pichia anomalus*	*phut*
47	STY21	*Pichia anomalus*	*phut*
49	STY21	*Candida glabrata*	*thiat*
49	STY6	*Candida glabrata*	*thiat*
50	STY24	*Pichia anomalus*	*thiat*
51	STY15	*Pichia anomalus*	*thiat*
52	STY12	*Debromyces*	*thiat*
53	STY3	*Debromyces castelii*	*thiat*
54	STY49	*Debromyces polymorphus*	*thiat*
55	STY49	*Candida glabrata*	*thiat*
56	STY6	*Pichia anomalus*	*thiat*
57	STY24	*Pichioa terricola*	*thiat*
59	STY15	*Saccharomycopsis fibuligera*	*thiat*
59	STY12	*Pichia anomalus*	*thiat*
60	STY3	*Pichia anomalus*	*thiat*

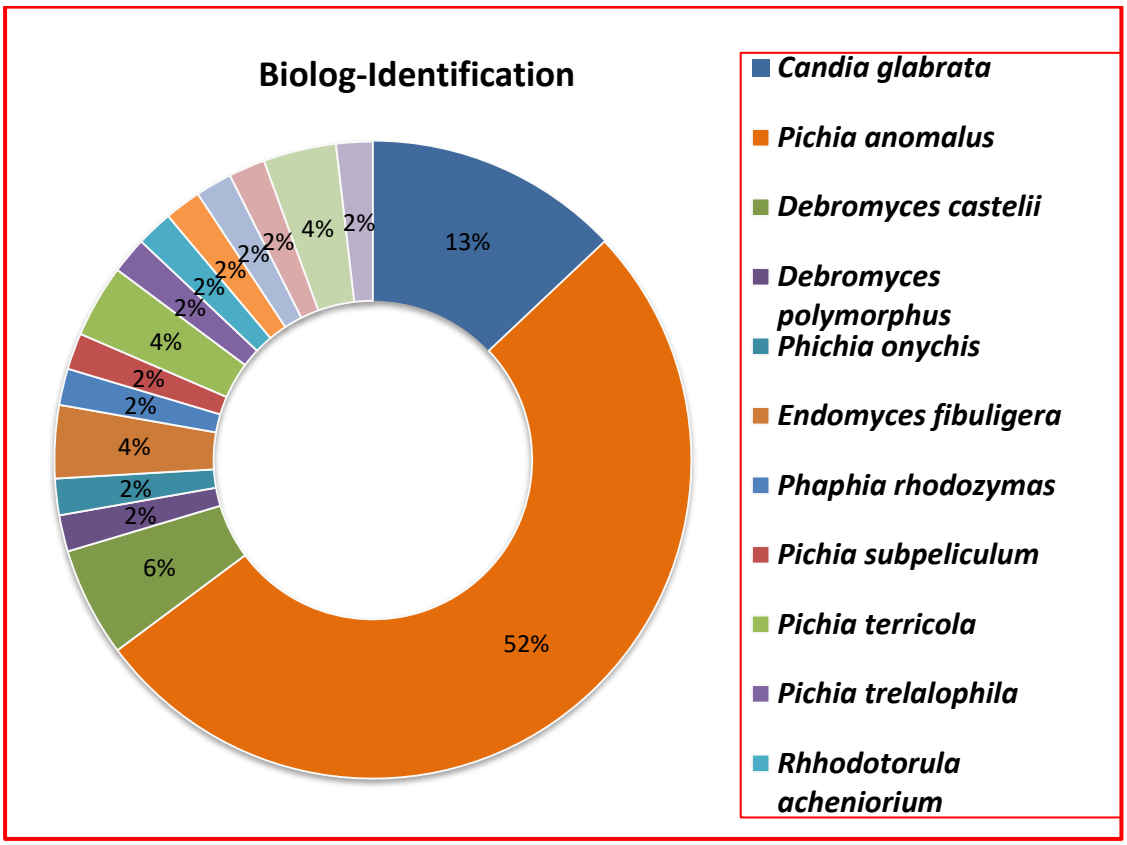


Figure 23. Distribution of yeasts in all the amylolytic starters of North East India

Table 44. Representative strains of Biolog identified yeasts isolated from amylolytic starters

Yeast Species	Probability (%)	Similarity	Distance	Status
Pichia anomala	0.943	0.683	4.185	Identified
Pichia terricola	0.974	0.768	3.182	Identified
Pichia sydowiorum	0.834	0.652	3.285	Identified
Pichia onychis	0.834	0.737	3.234	Identified
Pichia guillermondii	0.834	0.652	3.223	Identified
Pichia subpeliculum	0.834	0.734	3.764	Identified
Pichia trelalophila	0.834	0.794	3.234	Identified
Candia glabrata	0.834	0.786	3.864	Identified
Saccharomycopsis fibuligera	0.934	0.739	3.123	Identified
Zygosaccharomyces bailii	0.834	0.783	3.652	Identified
Phaffia rhodozyma	0.734	0.768	3.223	Identified
Debromyces	0.934	0.752	3.682	Identified
Debromyces castelii	0.834	0.754	3.285	Identified
Debromyces polymorphus	0.834	0.783	2.876	Identified
Issatchenkia orientalis	0.834	0.656	3.987	Identified
Saccharomyces cerevisiae	0.834	0.765	3.243	Identified
Rhodotolura bacarum	0.834	0.784	2.239	Identified
Rhodotorula aurantaea	0.834	0.618	2.285	Identified
Rhodotorula acheniorium	0.916	0.742	3.947	Identified

ITS-PCR

Out of 386 isolates, 46 representative isolates *Wickerhamomyces anomalus* (KY38, KY18, KY20, KY8, MY3, MY57, MY47, MY20, MY9, STY20, STY53, CHY22, CHX39, CHX26, CX44, CHY39, ASY4, ASY7, MY8, STY49, STY3, STY12, STY6, MY5, GMY46, GMY29, GMY5, GMY1, GM29) *Pichia anomala* (KY27, XTY20, MY6, GMY12), *Saccharomycopsis fibuligera* (XTY15, STY15, STY21, STY21), *Candida glabrata* (MY30, CHY28, AH45, KY45), *Pichia kudriavzevii* (HY7), *Pichia terricola* (STY24) (Table. 46) were further grouped based on colony appearance, cell shape, type of mycelia and ascospores, pellicle formation, nitrate reduction, and growth at 37 ^{o}C and 45 ^{o}C. Precisely, species level identification was done with molecular methods by ITS-region gene sequence analysis (Figure. 46). We

found that all cultures were identified in 06 species only as: *Wickerhamomyces anomalus, Pichia anomala, Saccharomycopsis fibuligera, Pichia terricola, Pichia kudriavzevii* and *Candida glabrata* (Figure. 37). The average distributions of yeasts in all amylolytic starters analysed by molecular tool presented in (Figure. 28 and 29). From the sequencing results of ITS-region gene; it was observed that species richness (R) was higher in *dawidim, hamei, thiat* than *marcha khekhrii, chowan* and *phut* (Table. 44). *Wickerhamomyces anomalus* was dominant in all starters. The Shanon index (H) of yeasts isolates was higher isolated from *dawidim* than *thiat, hamei, marcha, khekhrii, chowan, huamo* and *phut* (Table. 45). The highest Shanon index was observed in *dawidim* samples and lowest was observed in *phut* samples (Table. 44).

The diversity of yeasts associated with traditionally prepared amylolytic starters was investigated by using culture dependent molecular tool ITS-PCR. We identified *Wickerhamomyces anomalus, Pichia anomala, Saccharomycopsis fibuligera, Pichia terricola Pichia kudriavzevii* and *Candida glabrata* by targeting the ITS gene of 18SrRNA (Table. 45). The average distributions of yeasts in all eight amylolytic starters identified by ITS-PCR tools *Wickerhamomyces anomalus* (47.4%), *Pichia anomala* (13.4%), *Saccharomycopsis fibuligera* (5.0%), *Pichia terricola* (3.8%), *Pichia kudriavzevii* (7.9%) and *Candida glabrata* (18.8%) (Figure. 38, 39). From the sequencing results of ITS gene it was observed that species richness (R) is higher in *dawidim, hamei, thiat* than *marcha khekhrii, chowan* and *phut* (Table. 45). *Wickerhamomyces anomalus* was most dominant yeast species observed in all the amylolytic starters of North East India presented in (Table. 46). The Shanon index (H) of yeasts isolates were higher isolated from

dawidim than *thiat*, *hamei*, *marcha*, *khekhrii*, *chowan*, *huamo* and *phut* (Table. 45). The highest Shanon index was observed in the *dawdim* samples and lowest was observed in *phut* sample (Table. 45). ITS-PCR results showed the dominance of 32 starins of *Wickerhamomyces anomalus*, 4 strains of *Pichia anomala*, 4 strains of *Saccharomycopsis fibuligera*, 4 strains of *Candida glabrata* and 1 each strain of *Pichia terricola* and *Pichia kudriavzevii* (Figure.30). Molecular phylogenetic analysis of total 46 yeast isolates recovered from amylolytic starters based on ITS region sequencing. The bootstrap consensus tree derived with 1000 replicates to Neighbor-joining method and Kimura 2-parameter. Numbers on branches depict the percent occurrence of a given branch during 1000 replicates (Figure. 30).

Table 45. Molecular characterization and identification results of 46 yeast strains from amylolytic starters of North East India by PCR-ITS1-5.8S ITS2

Product	Isolate code	[a]AP	[b]H	[c]R	GenBank accession number	Species
Marcha	GM:29	554	0.642	2	KY605141	*Wickerhamomyces anomalus*
	GM:Y1	582	0.613		KY605153	*Wickerhamomyces anomalus*
	GM:Y5	548	0.623		KY605154	*Wickerhamomyces anomalus*
	GM:Y12	529	0.626		KY587129	*Pichia anomala*
	GM:Y29	483	0.625		KY587130	*Wickerhamomycesanomalus*
	GM:Y46	604	0.623		KY587131	*Wickerhamomyces anomalus*
	M:Y5	658	0.622		KY605150	*Wickerhamomyces anomalus*
Thiat	ST:Y21	793	6.000		KY605140	*Saccharomycopsis fibuligera*
	ST:Y6	705	0.911	3	KY605145	*Wickerhamomyces anomalus*
	ST:Y24	840	0.941		KY605146	*Pichia terricola*
	ST:Y15	624	0.921		KY605147	*Saccharomycopsis fibuligera*
	ST:Y12	702	0.901		KY605148	*Wickerhamomyces anomalus*
	ST:Y3	596	6.911		KY605149	*Wickerhamomyces anomalus*
	ST:Y49	661	0.921		KY626330	*Wickerhamomyces anomalus*
Hamei	M:Y8	661	0.911	3	KY587121	*Wickerhamomyces anomalus*
	HS:Y7	1031	0.921		KY626335	*Pichia kudriavzevii*
	AH:45	458	0.921		KY605155	*Candida glabrata*
	H:Y7	710	0.941		KY605152	*Pichia kudriavzevii*
Huamo	AS:Y3	515	0.441	1	KY587126	*Wickerhamomyces anomalus*
	AS:Y5	601	0.441		KY587127	*Wickerhamomyces anomalus*
	AS:Y7	594	0.401		KY587128	*Wickerhamomyces anomalus*
	AS:Y4	565	0.431		KY605162	*Wickerhamomyces anomalus*
Chowan	CH:Y28	801	0.621	2	KY605143	*Candida glabrata*
	CH:Y39	574	0.601		KY605144	*Wickerhamomyces anomalus*
	CX:44	258	0.621		KY605159	*Wickerhamomyces anomalus*

	CH:X26	594	0.611		KY605160	*Wickerhamomyces anomalus*
	CH:X39	918	0.631		KY626331	*Wickerhamomyces anomalus*
	CH:Y22	845	0.601		KY626334	*Wickerhamomyces anomalus*
Phut	ST:Y53	927	0.410	1	KY626332	*Wickerhamomyces anomalus*
	ST:Y20	919	0.400		KY626333	*Wickerhamomyces anomalus*
Dawdim	M:Y9	592	1.100	4	KY587136	*Wickerhamomyces anomalus*
	M:Y20	484	1.030		KY587137	*Wickerhamomyces anomalus*
	M:Y30	529	1.002		KY587138	*Candida glabrata*
	M:Y47	588	1.001		KY587139	*Wickerhamomyces anomalus*
	M:Y57	585	1.1 11		KY587140	*Wickerhamomyces anomalus*
	M:Y3	629	1.121		KY587119	*Wickerhamomyces anomalus*
	M:Y6	627	1.120		KY587120	*Pichia anomala*
	ST:Y15	692	1.120		KY605157	*Saccharomycopsis fibuligera*
	XT:Y20	610	1.131		KY605156	*Pichia anomala*
	XT:Y15	654	1.113		KY605147	*Saccharomycopsis fibuligera*
Khekhrii	K:Y8	558	0.630	2	KY605151	*Wickerhamomyces anomalus*
	K:Y20	589	0.600		KY605152	*Wickerhamomyces anomalus*
	K:Y18	529	0.601		KY587132	*Wickerhamomyces anomalus*
	K:Y27	599	0.611		KY587133	*Pichia anomala*
	K:Y38	604	0.620		KY587134	*Wickerhamomyces anomalus*
	K:Y45	599	0.612		KY587135	*Wickerhamomyces anomalus*

[a]AP, arbitary primers = sizes in base pairs; [b]H, Shannon's index; [c]R, Species richness.Only gene bank percent of strains with more than 90 % were shown in the Table.

Table 46. Gene bank accessions number of identified species of yeasts

Sl.	Isolate code	GenBank accession s	Identified Species	Samples
1	GM29	KY605141	*Wickerhamomyces anomalus*	*Marcha*
2	GMY1	KY605153	*Wickerhamomyces anomalus*	*Marcha*
3	GMY5	KY605154	*Wickerhamomyces anomalus*	*Marcha*
4	GMY12	KY587129	*Pichia anomala*	*Marcha*
5	GMY29	KY587130	*Wickerhamomyces anomalus*	*Marcha*
6	GMY46	KY587131	*Wickerhamomyces anomalus*	*Marcha*
7	MY5	KY605150	*Wickerhamomyces anomalus*	*Marcha*
8	STY21	KY605140	*Saccharomycopsis fibuligera*	*Thiat*
9	STY6	KY605145	*Wickerhamomyces anomalus*	*Thiat*
10	STY24	KY605146	*Pichia terricola*	*Thiat*
11	STY15	KY605147	*Saccharomycopsis fibuligera*	*Thiat*
12	STY12	KY605148	*Wickerhamomyces anomalus*	*Thiat*
13	STY3	KY605149	*Wickerhamomyces anomalus*	*Thiat*
14	STY49	KY626330	*Wickerhamomyces anomalus*	*Thiat*
15	MY8	KY587121	*Wickerhamomyces anomalus*	*hamei*
16	HSY7	KY626335	*Pichia kudriavzevii*	*hamei*
17	AH45	KY605155	*Candida glabrata*	*hamei*
18	HY7	KY605142	*Pichia kudriavzevii*	*hamei*
19	ASY3	KY587126	*Wickerhamomyces anomalus*	*humao*
20	ASY5	KY587127	*Wickerhamomyces anomalus*	*humao*
21	ASY7	KY587128	*Wickerhamomyces anomalus*	*huamo*
22	ASY4	KY605162	*Wickerhamomyces anomalus*	*huamo*
23	CHY28	KY605143	*Candida glabrata*	*chowan*
24	CHY39	KY605144	*Wickerhamomyces anomalus*	*chowan*
25	CX44	KY605159	*Wickerhamomyces anomalus*	*chowan*
26	CHX26	KY605160	*Wickerhamomyces anomalus*	*chowan*
27	CHX39	KY626331	*Wickerhamomyces anomalus*	*chowan*
28	CHY22	KY626334	*Wickerhamomyces anomalus*	*chowan*
29	STY53	KY626332	*Wickerhamomyces anomalus*	*phut*
30	STY20	KY626333	*Wickerhamomyces anomalus*	*phut*
31	MY9	KY587136	*Wickerhamomyces anomalus*	*dawdim*
32	MY20	KY587137	*Wickerhamomyces anomalus*	*dawdim*
33	MY30	KY587138	*Candida glabrata*	*dawdim*
34	MY47	KY587139	*Wickerhamomyces anomalus*	*dawdim*
35	MY57	KY587140	*Wickerhamomyces anomalus*	*dawdim*
36	MY3	KY587119	*Wickerhamomyces anomalus*	*dawdim*
37	MY6	KY587120	*Pichia anomala*	*dawdim*
38	STY15	KY605157	*Saccharomycopsis fibuligera*	*dawdim*
39	XTY20	KY605156	*Pichia anomala*	*dawdim*

40	XTY15	KY605147	*Saccharomycopsis fibuligera*	*dawdim*
41	KY8	KY605151	*Wickerhamomyces anomalus*	*khekhrii*
42	KY20	KY605152	*Wickerhamomyces anomalus*	*khekhrii*
43	KY18	KY587132	*Wickerhamomyces anomalus*	*khekhrii*
44	KY27	KY587133	*Pichia anomala*	*khekhrii*
45	KY38	KY587134	*Wickerhamomyces anomalus*	*khekhrii*
46	KY45	KY587135	*Candida glabrata*	*khekhrii*

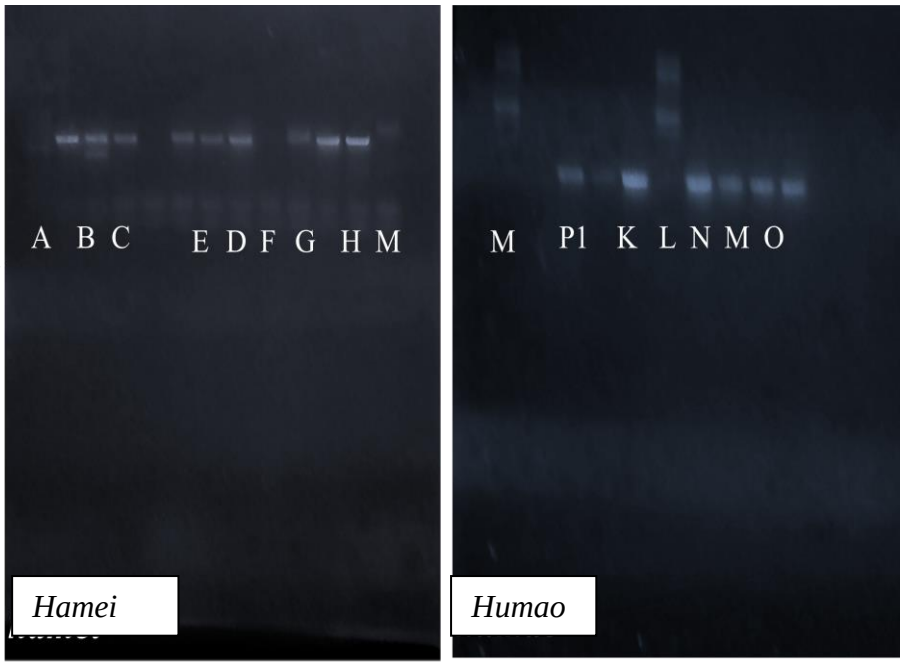


Figure 24.The representative yeasts isolates from *hamei* HSY7, AH45, HY7 and *humao* ASY3, ASY3, ASY5, ASY5, ASY4 are amplified for the ITS1 gene by using primer ITS1 and ITS4.

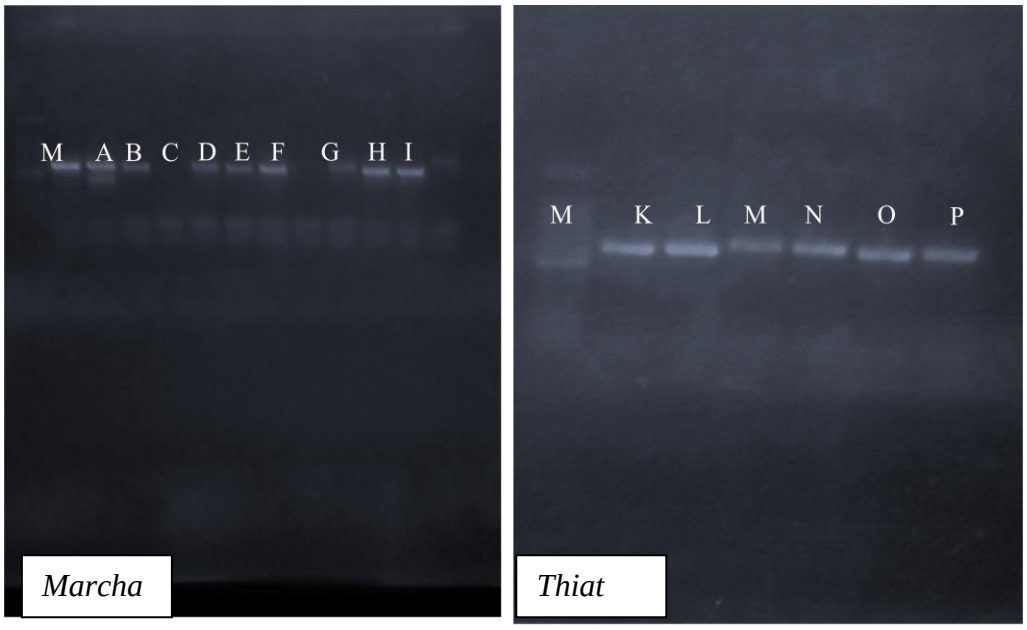


Figure 25. The representative yeasts isolates from *marcha* GM29, GMY1, GMY5, GMY12, GMY29, GMY46, MY15 and from *thiat* STY21, STY6, STY24, STY15, STY12, STY3, STY49 amplified for the ITS gene by using primers ITS1 and ITS4.

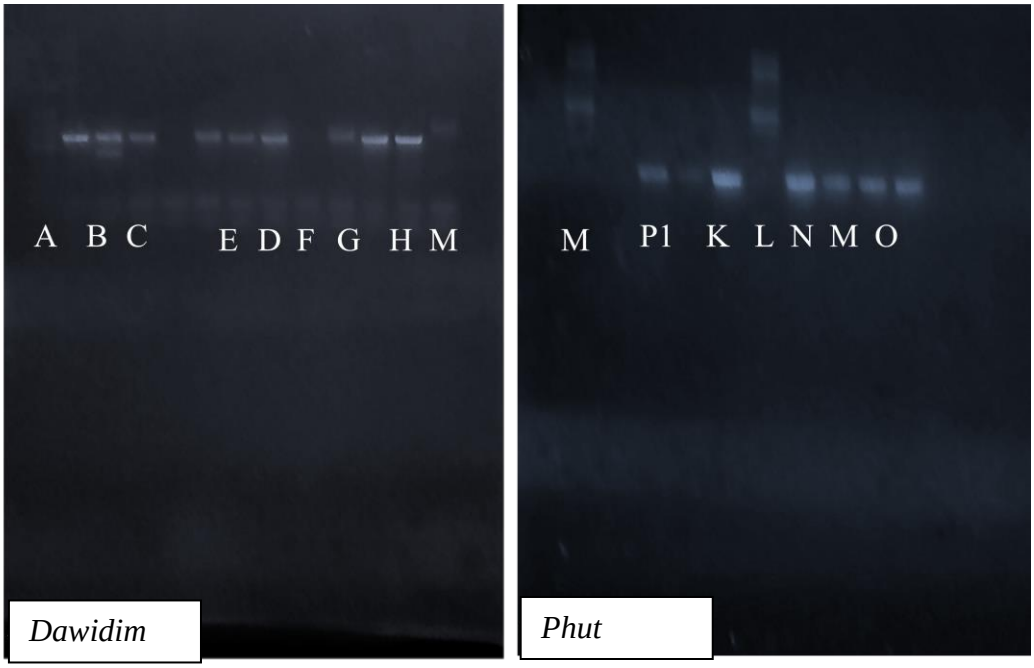


Figure 26.The representative yeasts isolates from *dawidim* MY9, MY20, MY30,MY47, MY57, MY3,MY3, MY6, STY15, XTY20, STY15 and *phut* STY20 and STY49 amplified for the ITS gene by using primers ITS1 and ITS4.

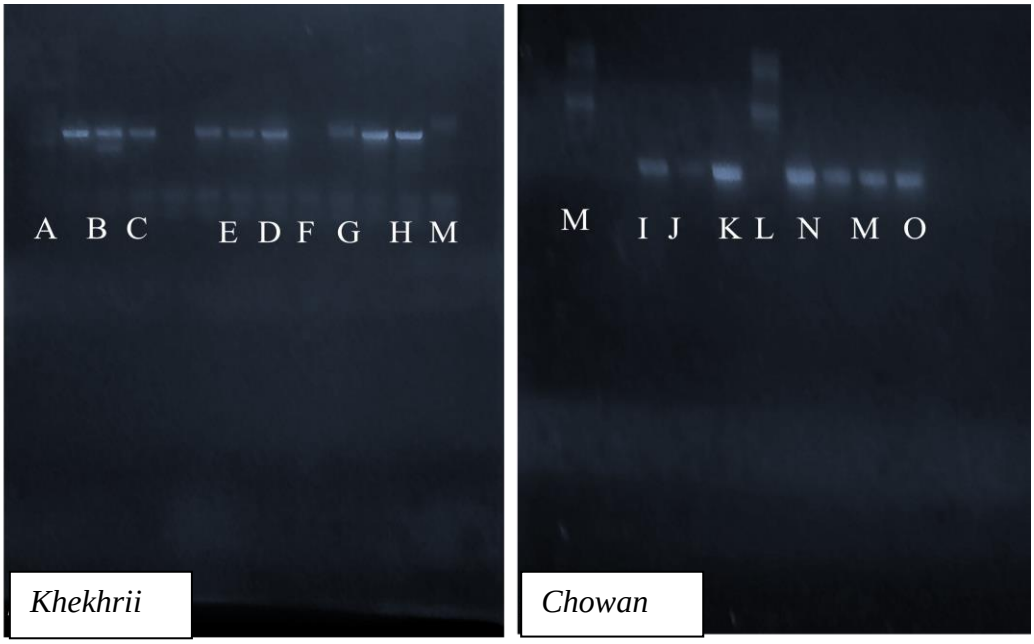


Figure 27.The representative yeasts isolates from *khekhrii* KY8, KY20, KY18, KY27, KY38, KY45 and *chowan* CHY28, CHY39, CX44, CHX26, CHX39, CHY22 and amplified for the ITS gene by using primer ITS1 and ITS4.

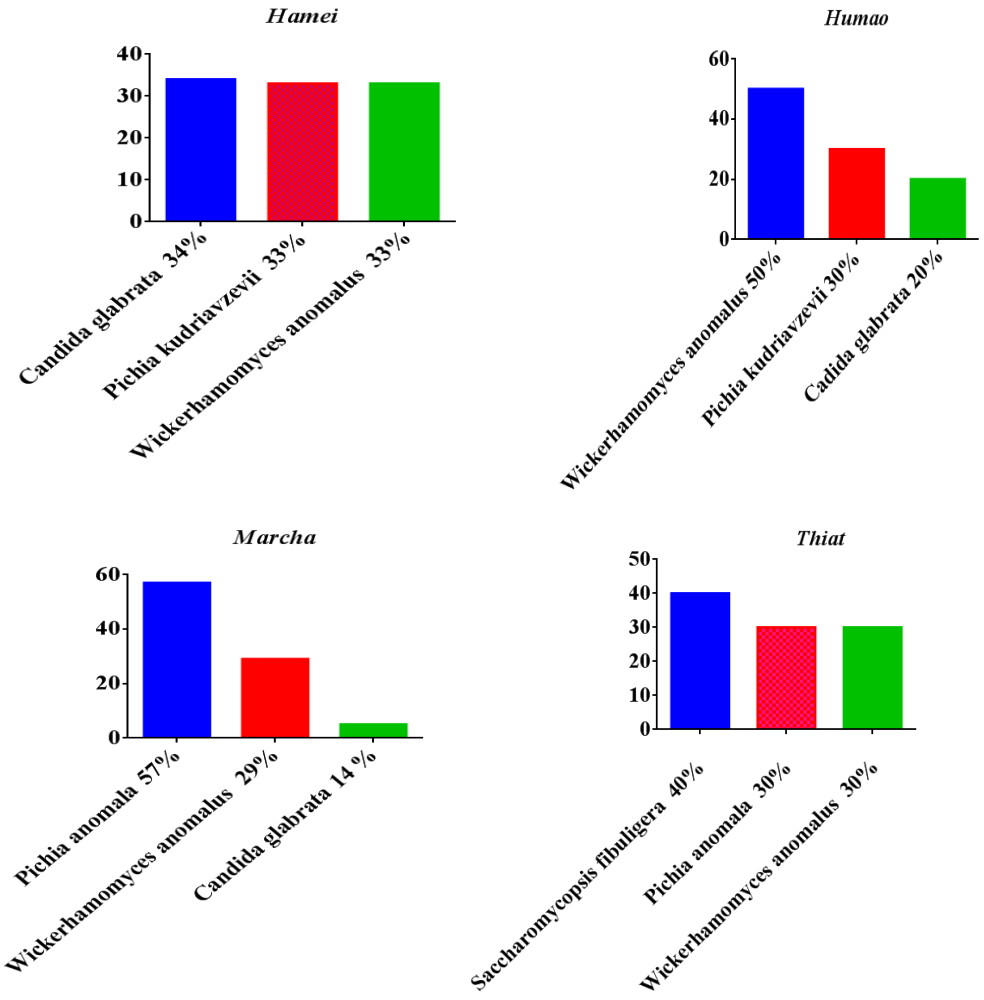


Figure 28. Percentage distribution of yeasts in *hamei*, *humao*, *marcha* and *thiat*.

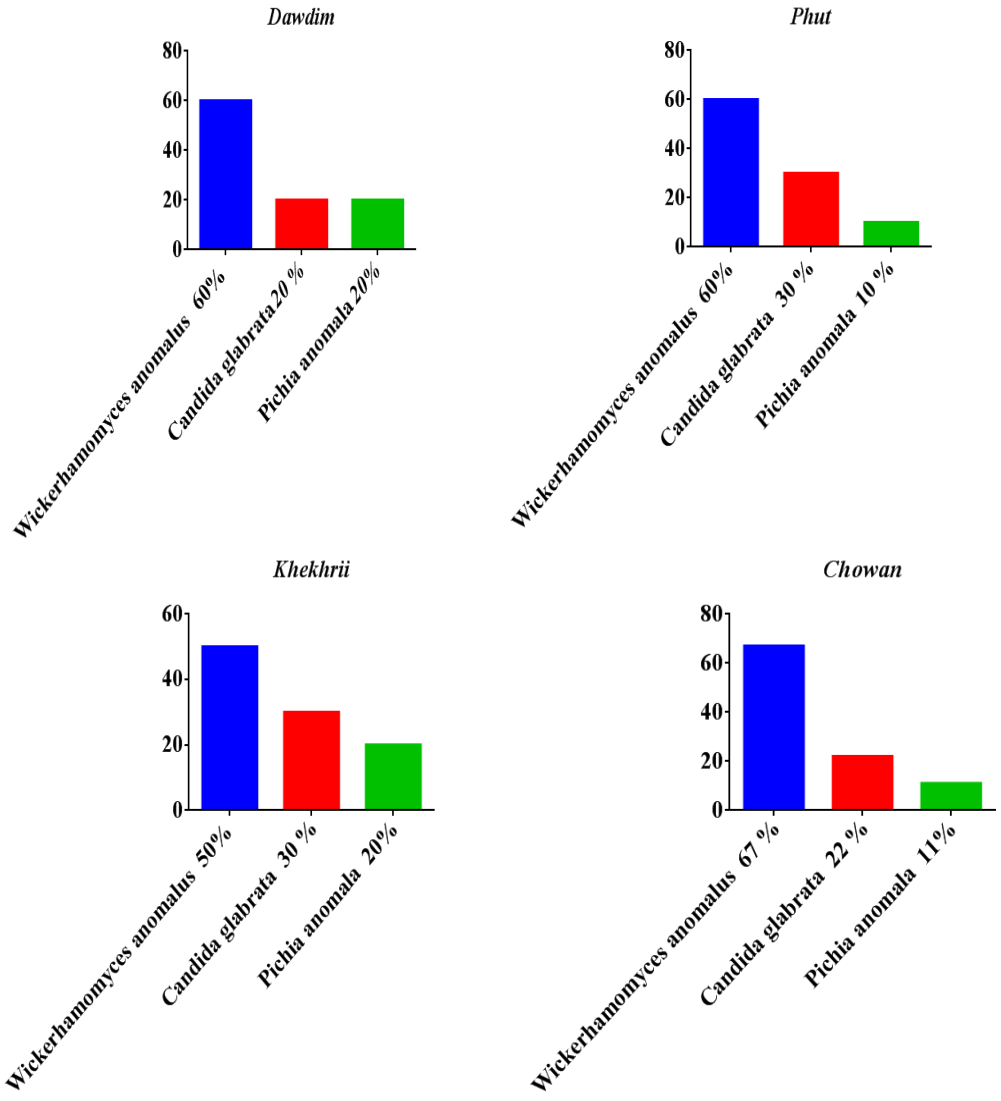


Figure 29.Percentage distribution of yeasts in *dawdim*, *phut*, *khekhrii* and *chowan*.

Table 47. Molecular characterization and identification results of 46 yeast strains from amylolytic starters of North East India by ITS-PCR

Product	Isolate code	[a]AP	[b]H	[c]R	GenBank accession number	Species
Marcha	GM:29	554	0.642	2	KY605141	*Wickerhamomyces*

						anomalus
	GM:Y1	582	0.613		KY605153	*Wickerhamomyces anomalus*
	GM:Y5	548	0.623		KY605154	*Wickerhamomyces anomalus*
	GM:Y12	529	0.626		KY587129	*Pichia anomala*
	GM:Y29	483	0.625		KY587130	*Wickerhamomycesanomalus*
	GM:Y46	604	0.623		KY587131	*Wickerhamomyces anomalus*
	M:Y5	658	0.622		KY605150	*Wickerhamomyces anomalus*
Thiat	ST:Y21	793	6.000		KY605140	*Saccharomycopsis fibuligera*
	ST:Y6	705	0.911	3	KY605145	*Wickerhamomyces anomalus*
	ST:Y24	840	0.941		KY605146	*Pichia terricola*
	ST:Y15	624	0.921		KY605147	*Saccharomycopsis fibuligera*
	ST:Y12	702	0.901		KY605148	*Wickerhamomyces anomalus*
	ST:Y3	596	6.911		KY605149	*Wickerhamomyces anomalus*
	ST:Y49	661	0.921		KY626330	*Wickerhamomyces anomalus*
Hamei	M:Y8	661	0.911	3	KY587121	*Wickerhamomyces anomalus*
	HS:Y7	1031	0.921		KY626335	*Pichia kudriavzevii*
	AH:45	458	0.921		KY605155	*Candida glabrata*
	H:Y7	710	0.941		KY605152	*Pichia kudriavzevii*
Huamo	AS:Y3	515	0.441	1	KY587126	*Wickerhamomyces anomalus*
	AS:Y5	601	0.441		KY587127	*Wickerhamomyces*

						anomalus
	AS:Y7	594	0.401		KY587128	*Wickerhamomyces anomalus*
	AS:Y4	565	0.431		KY605162	*Wickerhamomyces anomalus*
Chowan	CH:Y28	801	0.621	2	KY605143	*Candida glabrata*
	CH:Y39	574	0.601		KY605144	*Wickerhamomyces anomalus*
	CX:44	258	0.621		KY605159	*Wickerhamomyces anomalus*
	CH:X26	594	0.611		KY605160	*Wickerhamomyces anomalus*
	CH:X39	918	0.631		KY626331	*Wickerhamomyces anomalus*
	CH:Y22	845	0.601		KY626334	*Wickerhamomyces anomalus*
Phut	ST:Y53	927	0.410	1	KY626332	*Wickerhamomyces anomalus*
	ST:Y20	919	0.400		KY626333	*Wickerhamomyces anomalus*
Dawdim	M:Y9	592	1.100	4	KY587136	*Wickerhamomyces anomalus*
	M:Y20	484	1.030		KY587137	*Wickerhamomyces anomalus*
	M:Y30	529	1.002		KY587138	*Candida glabrata*
	M:Y47	588	1.001		KY587139	*Wickerhamomyces anomalus*
	M:Y57	585	1.111		KY587140	*Wickerhamomyces anomalus*
	M:Y3	629	1.121		KY587119	*Wickerhamomyces anomalus*
	M:Y6	627	1.120		KY587120	*Pichia anomala*
	ST:Y15	692	1.120		KY605157	*Saccharomycopsis*

						fibuligera
	XT:Y20	610	1.131		KY605156	*Pichia anomala*
	XT:Y15	654	1.113		KY605147	*Saccharomycopsis fibuligera*
Khekhrii	K:Y8	558	0.630	2	KY605151	*Wickerhamomyces anomalus*
	K:Y20	589	0.600		KY605152	*Wickerhamomyces anomalus*
	K:Y18	529	0.601		KY587132	*Wickerhamomyces anomalus*
	K:Y27	599	0.611		KY587133	*Pichia anomala*
	K:Y38	604	0.620		KY587134	*Wickerhamomyces anomalus*
	K:Y45	599	0.612		KY587135	*Wickerhamomyces anomalus*
[a]AP, arbitary primers = sizes in base pairs; [b]H, Shannon's index; [c]R, Species richness. Only gene bank percent of strains with more than 90 % were shown in the Table.						

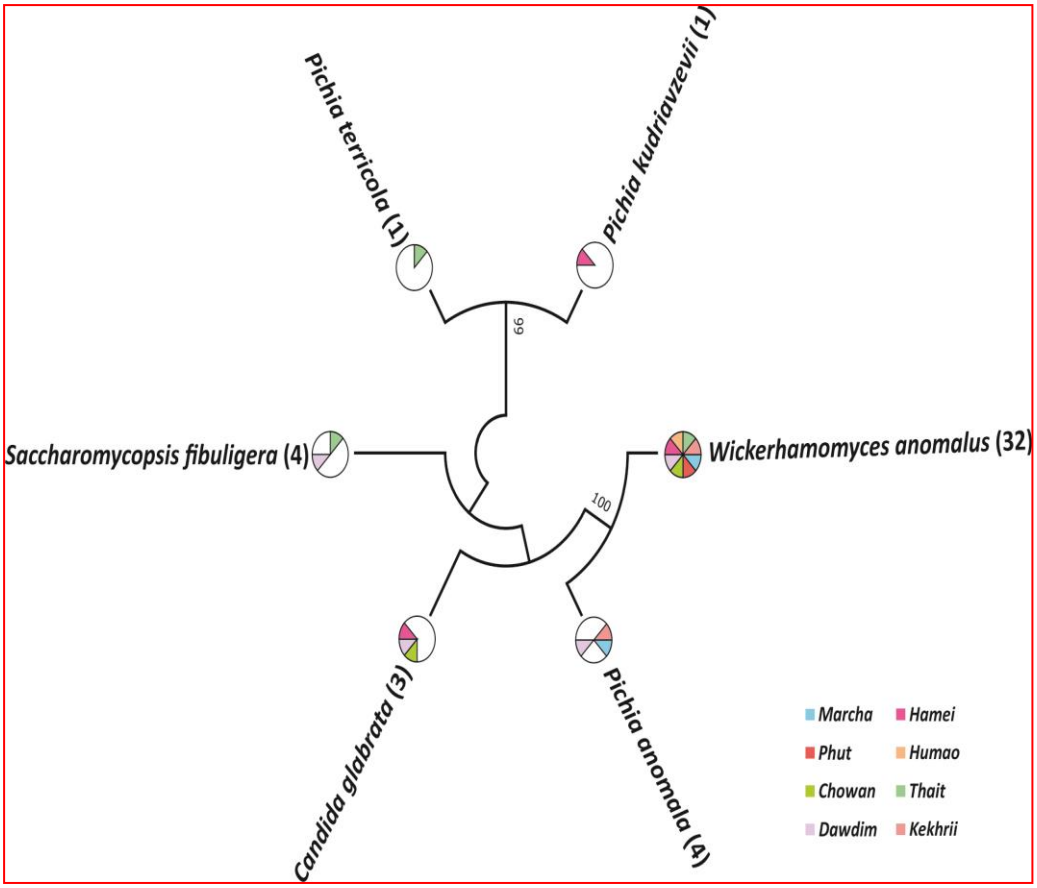


Figure 30**.** Molecular phylogenetic analysis of total 46 yeast isolates recovered from amylolytic starters based on ITS region sequencing. The bootstrap consensus tree derived with 1000 replicates to Neighbor-joining method and Kimura 2-parameter. Numbers on branches depict the percent occurrence of a given branch during 1000 replicates. The origin distribution patterns of these isolates were depicted in subsequent pi-charts.

CULTURE INDEPENDENT IDENTIFICATION RESULTS

PCR-DGGE ANALYSIS

In this study all eight amylolytic starters of North East India viz., *Marcha, humao, hamei, thiat, phut, khekhrii, chowan* and *dawdim* (Table. 1) were invstigated to reveal the complete yeasts and molds communities by using the PCR-DGGE technique. In the present study we targeted D1 and D2 domain of 26S rRNA gene (large ribosomal subunit) of yeast and mold from forty samples of amylolytic starters using PCR-DGGE fingerprint analysis. All the 40 genomic DNA extracterd from eight different amylolytic starters were purified and checked its purity on 1.5 percent agaarose gel (Figure. 31). We used NL-1 forward primer and a new LS2 reverse primer to amplify the portion of 26S rRNA gene. These primers amplify a product of approximately 250 bp covering most of the D1 expansion loop (Figure 32). In PCR-DGGE fingerprint, diversity map distributions in the form of band patterns of yeasts and molds had been observed in different starter cultures (Figure 33-46). Total 202 DGGE bands were selected on the basis of visualizing the prominent and differential band patterns inside the gels, after analysis of raw sequenced data with the help of BLAST comparison in GenBank as presented in (Table 39). More than 98 % similar identity with the closest species of yeasts and molds has different phylum and genus level distribution pattern in different starters (Table. 40). Interestingly, we observed the distinct species are more than the shared species and *Phut* were found to have high diversity among the tested starter cultures (Figure. 37). All these different techniques revealed the diversity and their differences of mycobiome species in different starter cultures (Figure. 50). Notably, the

average distributions of yeasts in all amylolytic starters were summarised in (figure. 38) as *Saccharomyces cerevisiae* (16.5%), *Saccharomycopsis fibuligera* (15.3%), *Wickerhamomyces anomalus* (11.3%), *Sm. Malanga* (11.7%), *Kluyveromyces marxianus* (5.3%), *Meyerozyma* sp. (2.7%), *Candida glabrata* (2.7%), *Saccharomyces* sp. (1.3%), *Hyphopichia burtonii* (1.2%), *Schwanniomyces occidentalis* (1.1%), *Pichia kudriavzevi* (1.0%), *Torulaspora delbrueckii* (1.0%), *Zygosaccharomyces bailii* (1.0%), *Pichia guilliermondii* (1.0%), *Candida parapsilosis* (0.4%), *Komagataella pastoris* (0.3%), *Sacch. capsularis* (0.6%), *S. Paradoxus* (0.6%), and *C. tropicalis* (0.1%). Similarly, the average distributions of molds in amylolytic starters were *Aspergillus penicillioides* (5.0%), *Rhizopus oryzae* (3.3%), sub-phyllum: *Mucoromycotina* (2.1%), *Cryptococcus amylolentus* (1.7%), *Xerochrysium dermatitidis* (1.6%), *Aspergillusoryzae* (1.3%), *Neosartoryafischeri* (0.8%), *A.proliferans* (0.6%), *Chrysozyma griseoflava* (0.6%), *Stilbocrea*sp. (0.6%), *Mucor circinelloides* (0.5%), *Aureobasidium pullulans* (0.4%) and *Xeromyce sbisporus* (0.3%). The complete genus distribution of all yeasts analysed by the PCR-DGGE techniquies are showed in (figure. 39). This is the first report on fugal communities of traditionally prepared amylolytic starters using PCR-DGGE technique.

Yeast and mold distribution in *marcha* of Sikkim*:* The DGGE bands of DNA isolated from *marcha* samples indicated by the letters (M1-M5) were excised, re-amplified and subjected to sequencing (Figure 35). The sequencing results of *marcha* showed *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera*, *Saccharomycopsis malanga*, *Rhizopus oryzae*,

Meyerozyma sp., *Wickerhamomyces anomalus*, *Candida tropicalis*, *Pichia guilliermondii*, *Candida glabrata*, *Pichia kudriavzevi proliferans* (Figure. 50). The phylum level distribution of yeast and molds diversity in amylolytic starter, *marcha* (Ascomycota 93.3%, Zygomycota 6.7%) (Figure. 38).

Yeast and mold distribution in *khekhrii* of Nagaland: The DGGE bands of DNA isolated from *kekhrii* indicated by the letters (K1-K5) were excised, re-amplified and subjected to sequencing (Figure 35). The PCR-DGGE sequencing results of *khekhrii* samples showed the dominance of *Saccharomycopsis malanga*, *Kluyveromyces marxianus*, *Saccharomycopsis fibuligera*, *Cryptococcus amylolentus proliferans* (Figure. 50). The phylum level distribution of yeast and molds diversity in amylolytic starter, *khekhrii* (Ascomycota 100 %) (Figure. 38).

Yeast and mold distribution in *thiat* of Meghalaya*:* The DGGE bands of DNA isolated from *thiat* samples indicated by the letters (T1-T5) were excised, re-amplified and subjected to sequencing (Figure. 36). The PCR-DGGE sequencing results of *thiat* showed the *Schwanniomyces occidentalis*, *uncultured fungus*, *Saccharomyces cerevisiae*, *Rhizopus oryzae*, *Torulaspora delbrueckii*, *Saccharomyces* DGGE band, *Zygosaccharomyces bailii*, *Aspergillus penicillioides proliferans* (Figure. 50). The phylum level distribution of yeast and molds diversity in amylolytic starter, *thiat* (Ascomycota 90.0 %, Zygomycota 10.0%) (Figure. 38).

Yeast and mold distribution in *chowan* of Tripura*:* The PCR-DGGE bands of DNA isolated from *chowan* indicated by the letters (C1-C5) were excised, re-amplified and subjected to sequencing (Figure. 36). The sequencing results of *Chowan* showed the dominance of yeasts and moulds *Saccharomycopsis fibuligera*, *Wickerhamomyces anomalus*, *Hyphopichia burtonii*, *Meyerozyma sp*, *Saccharomyces cerevisiae*, *Xerochrysium dermatitidis*, *Aureobasidium pullulans, Aspergillus oryzae*, *Aspergillus penicillioides*, *Stilbocrea sp, Saccharomycopsis malanga proliferans* (Figure. 50). The phylum level distribution of yeast and molds diversity in amylolytic starter, *chowan* Ascomycota (100%) (Figure. 38).

Yeast and mold distribution in *hamei* of Manipur*:* The DGGE bands of DNA isolated from *hamei* indicated by the letters (H1-H5) were excised (Figure. 33), re-amplified and subjected to sequencing *Saccharomycopsis fibuligera*, *Rhizopus oryzae*, *Candida* sp., *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Saccharomyces paradoxus*, *Aspergillus* sp., *Saccharomycopsis capsularis*, *Saccharomyces cerevisiae proliferans* (Figure. 50). The phylum level distribution of yeast and molds diversity in amylolytic starter, *hamei* (Ascomycota 94.7%, Zygomycota 5.3%) (Figure. 38).

Yeast and mold distribution in *humao* of Assam: The DGGE bands of DNA isolated from *humao* indicated by the letters (P1-P5) were excised, re-amplified and subjected to sequencing (Figure. 33). The PCR-DGGE sequencing results of *humao* samples showed the *Saccharomycopsis malanga*, *Rhizopus oryzae*, *Saccharomycopsis fibuligera*, *Neosartorya fischeri*,

Wickerhamomyces anomalus, *Meyerozyma* sp *proliferans* (Figure. 50). The phylum level distribution of yeast and molds diversity in amylolytic starter, *humao* (Ascomycota 92.8%, Zygomycota 6.2%) (Figure. 38).

Yeast and mold distribution in *dawdim* of Mizoram: The DGGE bands of the DNA isolated from *dawdim* indicated by the letters (D1-D5) were excised, re-amplified and subjected to sequencing (Figure. 34). The PCR-DGGE sequencing results showed the dominance of *Saccharomycopsis fibuligera*, *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, *Aspergillus penicillioides*, *Saccharomycopsis malanga*, *Meyerozyma* sp., *Chrysozyma griseoflava*, *Hyphopichia burtonii*, *Xeromyces bisporus*, *Aspergillus proliferans proliferans* (Figure. 40). The phylum level distribution of yeast and molds diversity in amylolytic starter, *dawidim* (Ascomycota 94.7%, Zygomycota 5.3%) (Figure. 38).

Yeast and mold distribution in *phut* of Arunachal Pradesh: The PCR-DGGE analysis of *phut* samples indicated by the letters (A1-A5) were excised, re-amplified and subjected to sequencing (Figure. 34). Distribution of yeast and molds in the *phut* samples are *Saccharomycopsis fibuligera*, *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, *Aspergillus penicillioides*, *Saccharomycopsis malanga*, *Meyerozyma* sp, *Chrysozyma griseoflava*, *Hyphopichia burtonii*, *Xeromyces bisporus*, *Aspergillus proliferans* (Figure. 40). The phylum level distribution of yeast and molds in amylolytic starter, *phut* (Ascomycota 94.1%, Zygomycota 5.9%) (Figure. 38).

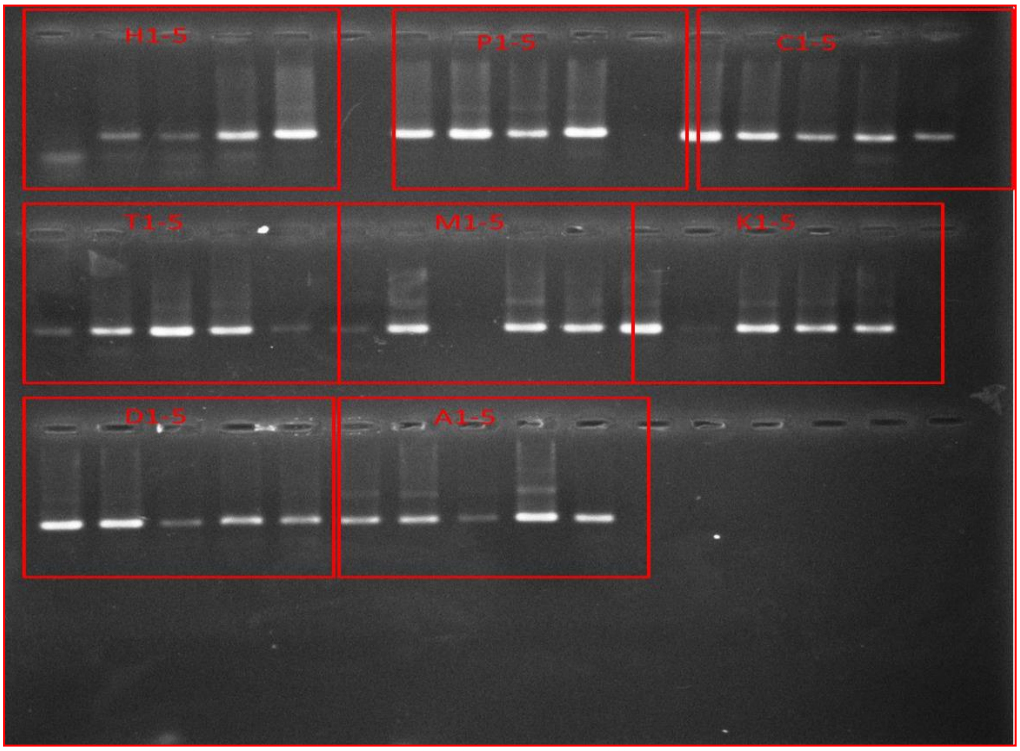


Figure 31. PCR products of all 40 extrcted genomic DNA observed on 1.5 % agaroge gel

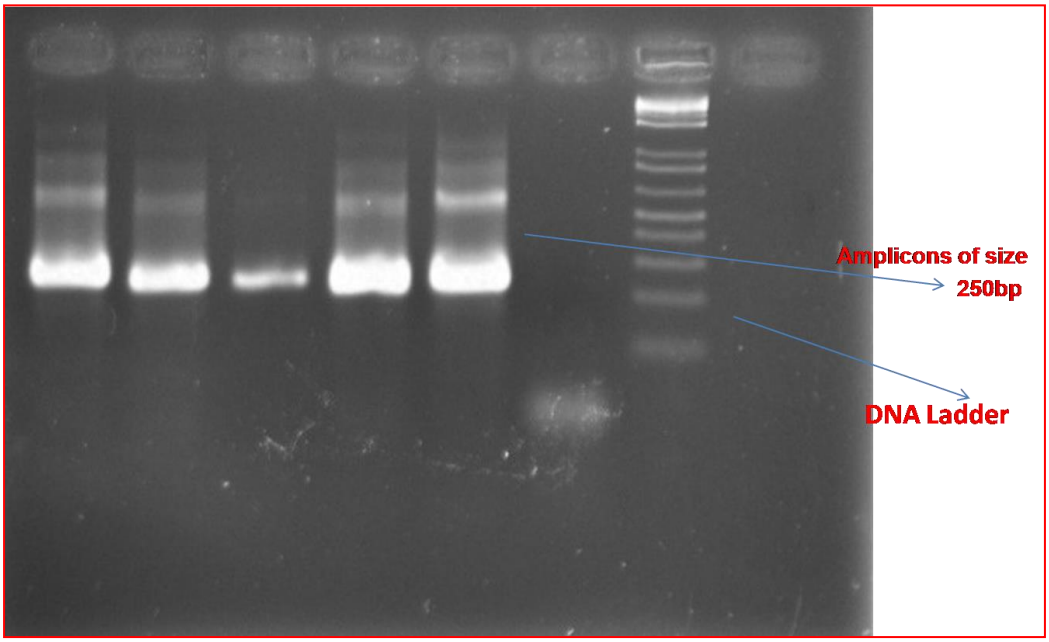


Figure 32. Gel image of amplicons of size 250bp amplified with forward primer NL1 and reverse primer LS2

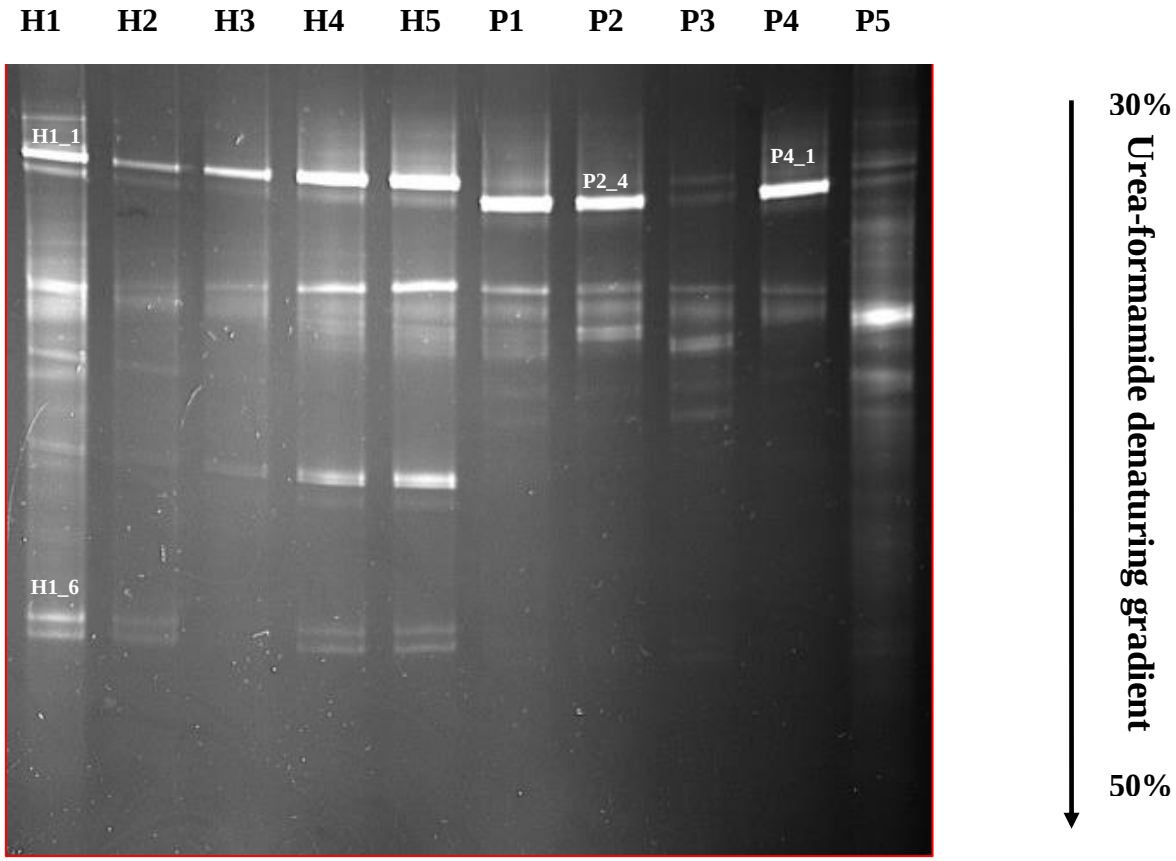


Figure 33. DGGE analysis of fungi in *hamei* and *humao.* Lanes H1–H5 refer to five samples of *hamei* and lanes P1-P5 refer to five samples of *huma,* respectively. Bands H1_1 and H1_6 correspond to the closest species of *Saccharomycopsis fibuligera, Aspergillus oryzae* and bands P2_4 and P4_1 correspond to the closest species of *Rhizopous oryzae* and *Saccharomycopsis malanga*, respectively.

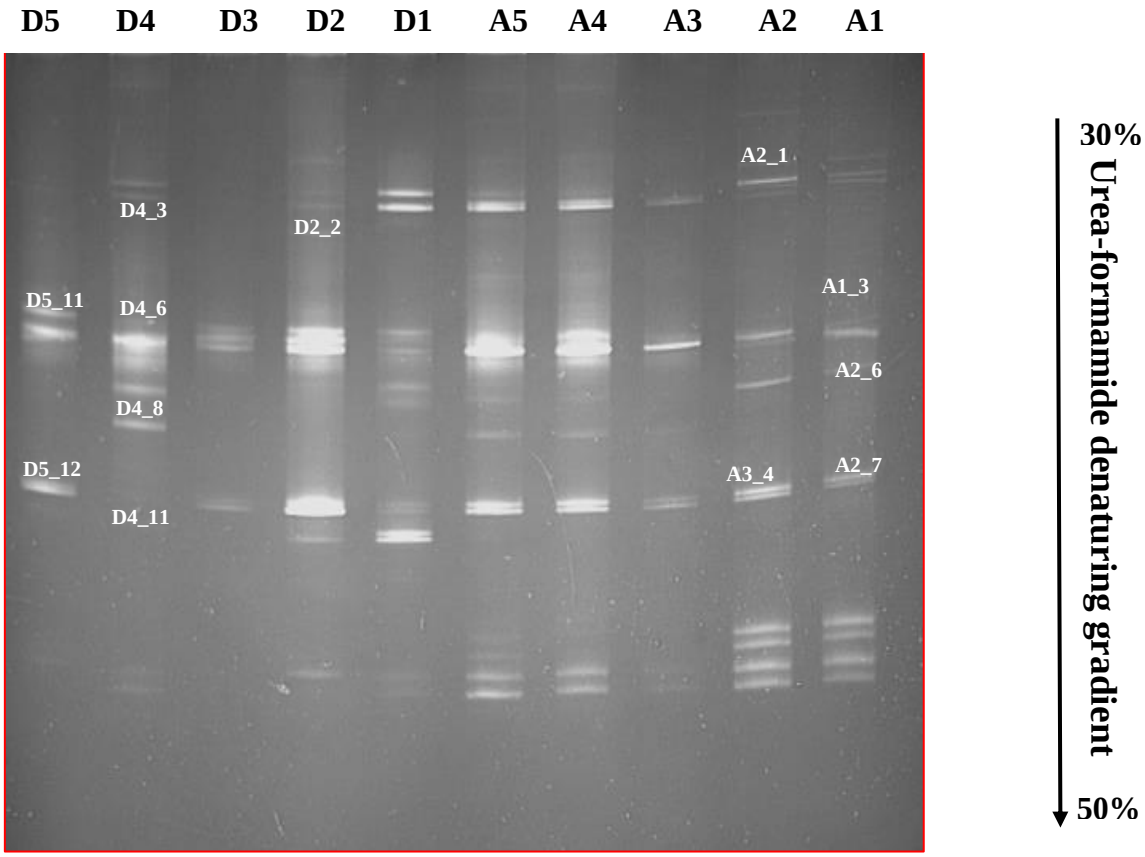


Figure 34. DGGE analysis of fungi in *dawdim* and *phut.* Lanes D1–D5 refer to five samples of *dawdim* and lanes A1-A5 refer to five samples of *phut*, respectively. Bands D2_2,D4_3,D4_6, D4_8, D4_11, D5_11,D5_12 correspond to the closest species of *Wickerhamomyces anomalus*, *Saccharomycopsis fibuligera*, *Hypopichia burtonii*,*Saccharomyces cerevisiae*,*Aspergillus penicillioides*and bands A2_1, A1_3, A2_6, A2_7, A3_4 correspond to the closest species of *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae* and *Hyphopichia burtonii*, respectively.

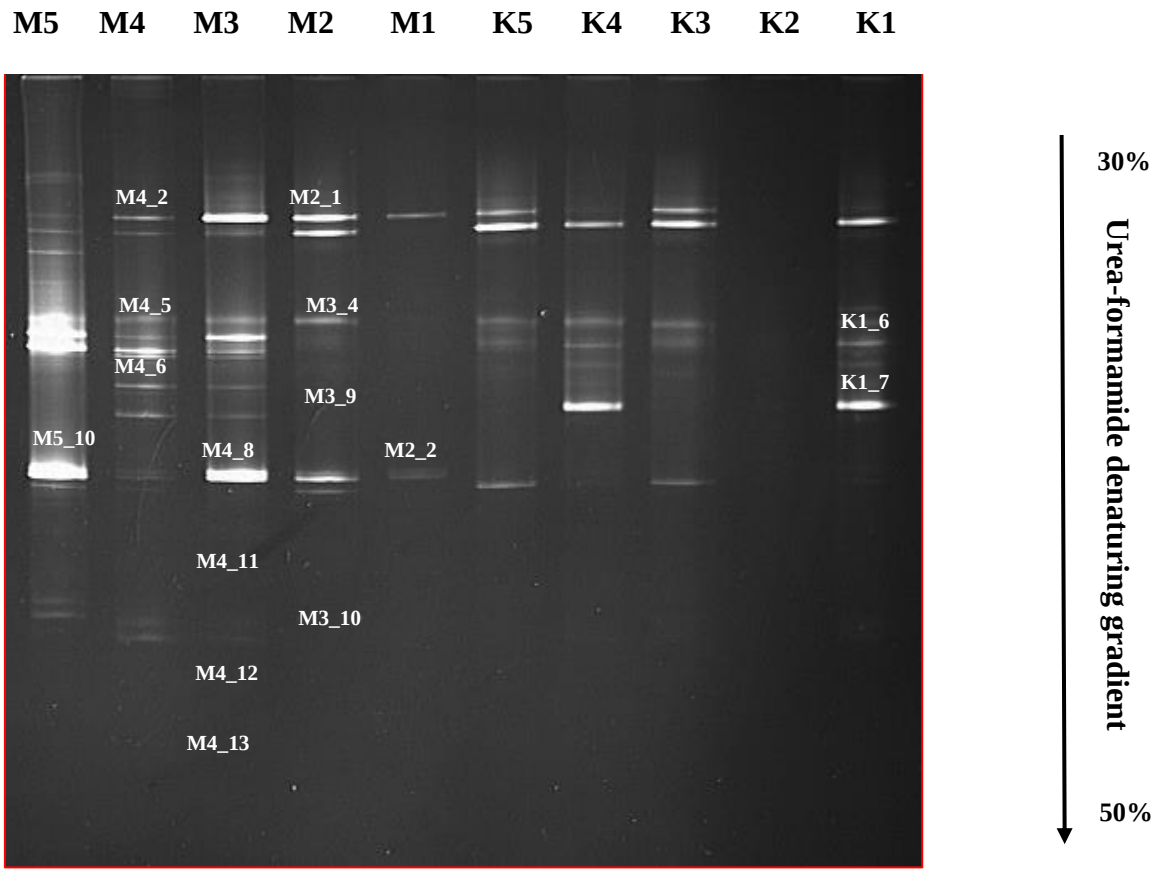


Figure 35. DGGE analysis of fungi in *marcha* and *khekhrii*. Lanes M1–M5 refer to five samples of *marcha* and lanes A1-A5 refer to five samples of *khekhrii*, respectively. Bands M4_2, M4_5, M4_6, M4_8, M4_11, M4_13, M2_2, M2_1, M3_4, M3_9, M3_10, M5_10 correspond to the closest species of *Saccharomycopsis malanga* (100%), *Wickerhamomyces anomalus*, *Candida tropicalis*, *Pichia guilliermondii*, *Candida glabrata*, *Pichia kudriavzevii*, *Rhizopus oryzae*, *Saccharomyces cerevisiae* and bands K1_6 and K1_7correspond to the closest species of *Kluyveromyces marxianus*, respectively.

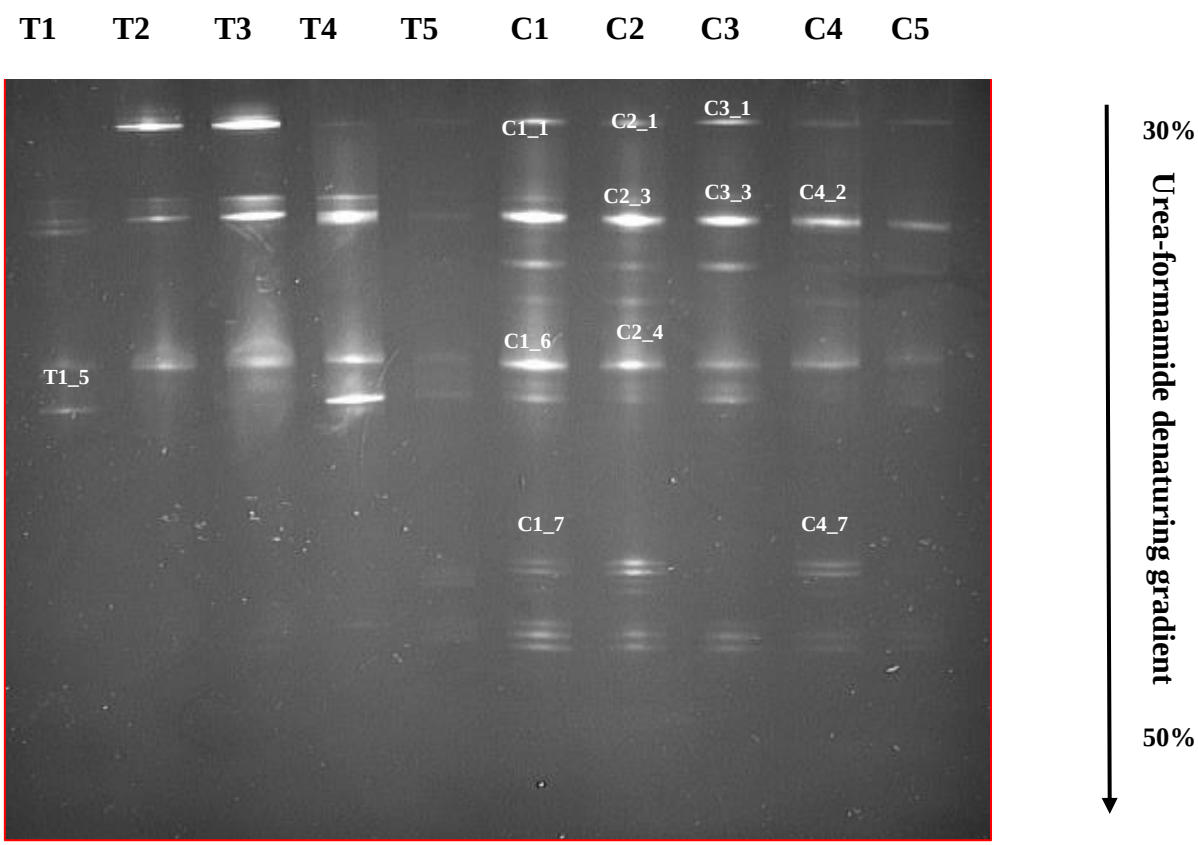


Figure 36. DGGE analysis of fungi in *thiat* and *chowan*. Lanes T1–T5 refer to five samples of *thiat* and lanes C1-C5 refer to five samples of *chowan*, respectively. The bands C1_1, C1_6, C1_7, C2_1, C2_3, C2_4, C3_1, C3_3, C4_3_C4_7 correspond to the closest species of *Saccharomycopsis fibuligera*, *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, *Aspergillus oryzae*, *Wickerhamomyces anomalus*, non-culturable *Hyphopichia*, *Saccharomycopsis fibuligera*, *Hyphopichiaburtonii*, *Wickerhamomyces anomalus*, *Aspergillus oryzae* and the bands T1_5 correspond to the closest species of *Saccharomyces cerevisiae*, respectively.

Table 48. Yeasts diversity analysed by PCR-DGGE method from different amylolytic starters of North East India

Sl. no	Band ID	GeneBank accessions	Blast hit results	Product size (bp)	% similarity	Samples
1	A1_2	KY594144	Uncultured fungus clone	188	98	*Phut*
2	A1_3	KY594145	*Saccharomyces cerevisiae*	188	100	*Phut*
3	A2_1	KY594146	*Saccharomycopsis fibuligera*	184	98	*Phut*
4	A2_2	KY594147	*Candida parapsilosis*	170	89	*Phut*
5	A2_3	KY594148	*Wickerhamomyces anomalus*	187	99	*Phut*
6	A2_4	KY594149	*Komagataella pastoris*	191	97	*Phut*
7	A2_5	KY594150	*Meyerozyma sp.*	188	99	*Phut*
8	A2_6	KY594151	*Pichia kudriavzevii*	178	100	*Phut*
9	A2_7	KY594152	*Pichia kudriavzevii*	177	99	*Phut*
10	A3_1	KY594153	*Rhizopus oryzae*	185	97	*Phut*
11	A3_2	KY594173	*Rhizopus oryzae*	183	95	*Phut*
12	A3_3	KY594154	*Wickerhamomyces anomalus*	201	99	*Phut*
13	A3_4	KY594155	*Saccharomyces cerevisiae*	185	100	*Phut*
14	A3_5	KY594156	*Uncultured Saccharomyces*	190	100	*Phut*
15	A4_1	KY594157	*Saccharomyces cerevisiae*	188	98	*Phut*
16	A4_3	KY594158	*Mucor circinelloides*	189	95	*Phut*
17	A4_4	KY594159	*Wickerhamomyces anomalus*	189	85	*Phut*
18	A4_5	KY594160	*Wickerhamomyces anomalus*	192	100	*Phut*
19	A4_6	KY594161	*Saccharomyces cerevisiae*	192	99	*Phut*
20	A4_7	KY594162	*Saccharomyces cerevisiae*	188	99	*Phut*
21	A4_8	KY594163	*Saccharomyces cerevisiae*	190	98	*Phut*
22	A5_1	KY594164	*Sampaiozyma vanillica*	190	89	*Phut*
23	A5_11	KY594171	*Botryosphaeria dothidea*	182	100	*Phut*
24	A5_12	KY594172	*Eurotiomycetes sp*	186	92	*Phut*
25	A5_2	KY594165	*Saccharomycopsis malanga*	191	98	*Phut*
26	A5_3	KY594166	*Rhizopus delemar*	185	98	*Phut*
27	A5_5	KY594167	*Debaryomyces*	175	91	*Phut*

			hansenii			
28	A5_6	KY594168	*Debaryomyces hansenii*	175	91	*Phut*
29	A5_7	KY594169	*Candida glabrata*	178	95	*Phut*
30	A5_8	KY594170	*Saccharomyces cerevisiae*	184	95	*Phut*
31	C1_1	KY594174	*Saccharomycopsis fibuligera*	171	100	*Chowan*
32	C1_10	KY594182	*Stilbocrea sp.*	187	98	*Chowan*
33	C1_11	KY594183	*Aureobasidium pullulans*	192	83	*Chowan*
34	C1_12	KY594184	*Aspergillus oryzae*	180	97	*Chowan*
35	C1_2	KY594175	*Wickerhamomyces anomalus*	165	98	*Chowan*
36	C1_3	KY594176	*Wickerhamomyces anomalus*	180	99	*Chowan*
37	C1_4	KY594177	*Hyphopichia burtonii*	182	92	*Chowan*
38	C1_5	KY594178	*Meyerozyma sp*	190	97	*Chowan*
39	C1_6	KY594179	*Wickerhamomyces anomalus*	184	100	*Chowan*
40	C1_7	KY594180	*Saccharomyces cerevisiae*	185	100	*Chowan*
41	C1_8	KY594181	*Xerochrysium dermatitidis*	173	97	*Chowan*
42	C2_1	KY594185	*Aspergillus penicillioides*	175	98	*Chowan*
43	C2_10	KY594193	*Aspergillus penicillioides*	147	98	*Chowan*
44	C2_11	KY594194	*Aspergillus penicillioides*	167	98	*Chowan*
45	C2_3	KY594186	*Wickerhamomyces anomalus*	194	100	*Chowan*
46	C2_4	KY594187	*Uncultured Hyphopichia*	193	98	*Chowan*
47	C2_5	KY594188	*Wickerhamomyces anomalus*	184	97	*Chowan*
48	C2_6	KY594189	*Wickerhamomyces anomalus*	192	100	*Chowan*
49	C2_7	KY594190	*Saccharomyces cerevisiae*	184	100	*Chowan*
50	C2_8	KY594191	*Xerochrysium dermatitidis*	183	98	*Chowan*
51	C2_9	KY594192	*Xerochrysium dermatitidis*	186	97	*Chowan*
52	C3_1	KY594195	*Saccharomycopsis fibuligera*	166	98	*Chowan*
53	C3_2	KY594196	*Wickerhamomyces anomalus*	183	99	*Chowan*

54	C3_3	KY594197	*Hyphopichia burtonii*	169	99	*Chowan*
55	C3_4	KY594198	*Wickerhamomyces anomalus*	181	98	*Chowan*
56	C3_5	KY594199	*Wickerhamomyces anomalu*	116	98	*Chowan*
57	C3_6	KY594200	*Uncultured Saccharomyces*	185	100	*Chowan*
58	C3_7	KY594201	*Stilbocrea sp*	182	97	*Chowan*
59	C3_8	KY594202	*Stilbocrea sp.*	154	88	*Chowan*
60	C3_9	KY594203	*Aspergillus oryzae*	170	97	*Chowan*
61	C4_1	KY594204	*Saccharomycopsis malanga*	164	96	*Chowan*
62	C4_2	KY594205	*Wickerhamomyces anomalus*	182	100	*Chowan*
63	C4_3	KY594206	*Wickerhamomyces anomalus*	159	99	*Chowan*
64	C4_5	KY594208	*Xerochrysium dermatitidis*	184	98	*Chowan*
65	C4_6	KY594209	*Aspergillus penicillioides*	184	97	*Chowan*
66	C4_7	KY594210	*Aspergillus penicillioides*	187	98	*Chowan*
67	C4-4	KY594207	*Xerochrysium dermatitidis*	161	99	*Chowan*
68	C5_1	KY594211	*Saccharomycopsis fibuligera*	175	85	*Chowan*
69	C5_2	KY594212	*Wickerhamomyces anomalus*	141	98	*Chowan*
70	D1_1	KY594101	*Saccharomycopsis fibuligera*	167	95	*Dawdim*
71	D1_3	KY594102	*Wickerhamomyces anomalus*	176	98	*Dawdim*
72	D1_4	KY594103	*Wickerhamomyces anomalus*	216	92	*Dawdim*
73	D1_5	KY594104	*Saccharomyces cerevisiae*	189	100	*Dawdim*
74	D1_6	KY594105	*Saccharomyces cerevisiae*	182	100	*Dawdim*
75	D1_7	KY594106	*Aspergillus penicillioides*	184	98	*Dawdim*
76	D1_8	KY594107	*Aspergillus penicillioides*	192	97	*Dawdim*
77	D2_1	KY594108	*Saccharomycopsis malanga*	188	95	*Dawdim*
78	D2_2	KY594109	*Wickerhamomyces anomalus*	180	100	*Dawdim*
79	D2_3	KY594110	*Wickerhamomyces anomalus*	181	99	*Dawdim*
80	D2_4	KY594111	*Meyerozyma sp.*	178	98	*Dawdim*

81	D2_5	KY594112	*Saccharomyces cerevisiae*	189	99	*Dawdim*
82	D2_6	KY594113	*Saccharomyces cerevisiae*	176	87	*Dawdim*
83	D2_7	KY594114	*Aspergillus penicillioides*	187	98	*Dawdim*
84	D2_8	KY594115	*Aspergillus penicillioides*	173	97	*Dawdim*
85	D3_1	KY594116	*Chrysozyma griseoflava*	162	83	*Dawdim*
86	D3_2	KY594117	*Wickerhamomyces anomalus*	177	97	*Dawdim*
87	D3_3	KY594118	*Meyerozyma sp.*	171	99	*Dawdim*
88	D3_4	KY594119	*Saccharomyces cerevisiae*	187	100	*Dawdim*
89	D3_5	KY594120	*Saccharomyces cerevisiae*	187	99	*Dawdim*
90	D3_6	KY594121	*Aspergillus penicillioides*	181	98	*Dawdim*
91	D4_1	KY594122	*Uncultured fungus*	197	87	*Dawdim*
92	D4_10	KY594131	*Xeromyces bisporus*	177	99	*Dawdim*
93	D4_11	KY594132	*Aspergillus penicillioides*	178	99	*Dawdim*
94	D4_12	KY594133	*Aspergillus penicillioides*	201	97	*Dawdim*
95	D4_2	KY594123	*Aspergillus penicillioides*	169	97	*Dawdim*
96	D4_3	KY594124	*Saccharomycopsis fibuligera*	172	99	*Dawdim*
97	D4_4	KY594125	*Uncultured soil fungus*	182	96	*Dawdim*
98	D4_5	KY594126	*Wickerhamomyces anomalus*	167	99	*Dawdim*
99	D4_6	KY594127	*Hyphopichia burtonii*	182	99	*Dawdim*
100	D4_7	KY594128	*Saccharomyces cerevisiae*	167	99	*Dawdim*
101	D4_8	KY594129	*Saccharomyces cerevisiae*	177	100	*Dawdim*
102	D4_9	KY594130	*Xeromyces bisporus*	183	99	*Dawdim*
103	D5_11	KY594142	*Aspergillus penicillioides*	190	99	*Dawdim*
104	D5_12	KY594143	*Aspergillus penicillioides*	189	98	*Dawdim*
105	D5_2	KY594134	*Aspergillus proliferans*	171	94	*Dawdim*
106	D5_3	KY594135	*Saccharomycopsis fibuligera*	169	98	*Dawdim*
107	D5_4	KY594136	*Wickerhamomyces anomalus*	179	99	*Dawdim*
108	D5_5	KY594137	*Wickerhamomyces*	163	99	*Dawdim*

			anomalus			
109	D5_6	KY594138	*Saccharomyces cerevisiae*	190	82	*Dawdim*
110	D5_7	KY594139	*Saccharomyces cerevisiae*	192	100	*Dawdim*
111	D5_8	KY594140	*Saccharomyces cerevisiae*	189	99	*Dawdim*
112	D5_9	KY594141	*Xeromyces bisporus*	187	99	*Dawdim*
113	H1_1	KY594045	*Saccharomycopsis fibuligera*	190	100	*Hamei*
114	H1_3	KY594046	*Saccharomycopsis malanga*	202	96	*Hamei*
115	H1_4	KY594047	*Rhizopus oryzae*	195	91	*Hamei*
116	H1_5	KY594048	*Candida sp.*	172	94	*Hamei*
117	H1_6	KY594049	*Aspergillus oryzae*	190	99	*Hamei*
118	H1_7	KY594050	*Saccharomyces cerevisiae*	188	95	*Hamei*
119	H2_1	KY594051	*Saccharomycopsis fibuligera*	186	100	*Hamei*
120	H3_1	KY594052	*Saccharomycopsis fibuligera*	182	100	*Hamei*
121	H4_1	KY594053	*Saccharomycopsis fibuligera*	191	99	*Hamei*
122	H4_2	KY594054	*Saccharomycopsis fibuligera*	190	99	*Hamei*
123	H4_3	KY594055	*Saccharomyces cf. cerevisiae/paradoxus*	188	95	*Hamei*
124	H4_5	KY594056	*Aspergillus sp.*	183	97	*Hamei*
125	H5_1	KY594057	*Saccharomycopsis fibuligera*	183	99	*Hamei*
126	H5_2	KY594058	*Saccharomycopsis capsularis*	173	89	*Hamei*
127	H5_3	KY594059	*Saccharomycopsis fibuligera*	186	94	*Hamei*
128	H5_4	KY594060	*Saccharomyces cerevisiae*	192	98	*Hamei*
129	H5_5	KY594061	*Saccharomyces cerevisiae*	155	83	*Hamei*
130	H5_6	KY594062	*Aspergillus sp.*	187	97	*Hamei*
131	H5_7	KY594063	*Aspergillus sp*	169	96	*Hamei*
132	K1_1	KY594080	*Saccharomycopsis malanga*	187	98	*Khekhrii*
133	K1_5	KY594081	*Kluyveromyces marxianus*	191	93	*Khekhrii*
134	K1_6	KY594082	*Kluyveromyces marxianus*	184	98	*Khekhrii*
135	K1_7	KY594083	*Kluyveromyces marxianus*	189	98	*Khekhrii*

136	K3_1	KY594084	*Saccharomycopsis fibuligera*	184	99	*Khekhrii*
137	K4_1	KY594085	*Saccharomycopsis malanga*	180	94	*Khekhrii*
138	K4_3	KY594086	*Torulaspora delbrueckii*	188	87	*Khekhrii*
139	K4_4	KY594087	*Kluyveromyces marxianus*	186	98	*Khekhrii*
140	K5_5	KY594088	*Candida glabrata*	156	85	*Khekhrii*
141	M1-2	KY594213	*Saccharomyces cerevisiae*	184	99	*Marcha*
142	M2_1	KY594214	*Saccharomycopsis fibuligera*	190	100	*Marcha*
143	M2_2	KY594215	*Saccharomycopsis malanga*	170	100	*Marcha*
144	M2_3	KY594216	*Saccharomycopsis fibuligera*	179	83	*Marcha*
145	M2_4	KY594217	*Saccharomyces cerevisiae*	187	100	*Marcha*
146	M2_5	KY594218	*Wickerhamomyces anomalus*	141	83	*Marcha*
147	M3_1	KY594219	*Saccharomycopsis fibuligera*	188	99	*Marcha*
148	M3_10	KY594226	*Saccharomyces cerevisiae*	185	100	*Marcha*
149	M3_2	KY594220	*Saccharomycopsis fibuligera*	167	99	*Marcha*
150	M3_4	KY594221	*Rhizopus oryzae*	188	98	*Marcha*
151	M3_5	KY594222	*Wickerhamomyces sp*	176	100	*Marcha*
152	M3_7	KY594223	*Hyphopichia burtonii*	188	96	*Marcha*
153	M3_8	KY594224	*Meyerozyma sp.*	198	98	*Marcha*
154	M3_9	KY594225	*Saccharomyces cerevisiae*	185	100	*Marcha*
155	M4_11	KY594236	*Candida glabrata*	150	98	*Marcha*
156	M4_12	KY594237	*Pichia kudriavzevii*	196	91	*Marcha*
157	M4_13	KY594238	*Pichia kudriavzevi*	193	99	*Marcha*
158	M4_2	KY594228	*Saccharomycopsis malanga*	173	99	*Marcha*
159	M4_3	KY594229	*Wickerhamomyces anomalus*	174	97	*Marcha*
160	M4_4	KY594230	*Candida tropicalis strain*	174	90	*Marcha*
161	M4_5	KY594231	*Wickerhamomyces anomalu*	185	100	*Marcha*
162	M4_6	KY594232	*Candida tropicalis*	184	99	*Marcha*
163	M4_7	KY594233	*Candida tropicalis*	198	85	*Marcha*
164	M4_8	KY594234	*Pichia guilliermondii*	188	98	*Marcha*
165	M4_9	KY594235	*Saccharomyces*	184	99	*Marcha*

			cerevisiae			
166	M4-1	KY594227	*Saccharomycopsis fibuligera*	185	99	*Marcha*
167	M5_1	KY594239	*Saccharomyces cerevisiae*	184	94	*Marcha*
168	M5_10	KY594246	*Saccharomyces cerevisiae*	195	100	*Marcha*
169	M5_2	KY594240	*Saccharomyces cerevisiae*	188	99	*Marcha*
170	M5_3	KY594241	*Wickerhamomyces anomalus*	177	87	*Marcha*
171	M5_4	KY594242	*Saccharomycopsis malanga*	190	98	*Marcha*
172	M5_5	KY594243	*Mucor zychae*	182	83	*Marcha*
173	M5_8	KY594244	*Saccharomyces cerevisiae*	200	99	*Marcha*
174	M5_9	KY594245	*Saccharomyces cerevisiae*	187	99	*Marcha*
175	P1_1	KY594064	*Saccharomycopsis malanga*	180	96	*Humao*
176	P1_2	KY594065	*Saccharomycopsis malanga*	182	98	*Humao*
177	P2_1	KY594066	*Saccharomycopsis malanga*	184	99	*Humao*
178	P2_4	KY594067	*Rhizopus oryzae*	189	99	*Humao*
179	P3_1	KY594068	*Saccharomycopsis fibuligera*	177	99	*Humao*
180	P3_2	KY594069	*Neosartorya fischeri*	176	78	*Humao*
181	P3_3	KY594070	*Wickerhamomyces anomalus*	177	94	*Humao*
182	P3_4	KY594071	*Wickerhamomyces anomalus*	176	97	*Humao*
183	P3_6	KY594072	*Meyerozyma sp.*	186	99	*Humao*
184	P4_1	KY594073	*Saccharomycopsis malanga*	175	99	*Humao*
185	P4_2	KY594074	*Saccharomycopsis malanga*	210	97	*Humao*
186	P4_3	KY594075	*Saccharomycopsis malanga*	175	96	*Humao*
187	P5_1	KY594076	*Saccharomycopsis fibuligera*	183	99	*Humao*
188	P5_2	KY594077	*Saccharomycopsis malanga*	182	98	*Humao*
189	P5_3	KY594078	*Wickerhamomyces anomalus*	175	97	*Humao*
190	P5_4	KY594079	*Wickerhamomyces anomalus*	181	83	*Humao*
191	T1_1	KY594089	*Schwanniomyces*	170	85	*Thiat*

			occidentalis			
192	T1_2	KY594090	*Uncultured fungus*	176	91	*Thiat*
193	T1_5	KY594091	*Saccharomyces cerevisiae*	151	100	*Thiat*
194	T2_1	KY594092	*Saccharomycopsis fibuligera*	174	98	*Thiat*
195	T3_1	KY594093	*Saccharomycopsis fibuligera*	209	99	*Thiat*
196	T3_2	KY594094	*Rhizopus oryzae*	185	90	*Thiat*
197	T3_3	KY594095	*Mucoromycotina sp.*	198	97	*Thiat*
198	T4_3	KY594096	*Mucoromycotina sp.*	205	97	*Thiat*
199	T4_4	KY594097	*Torulaspora delbrueckii*	190	87	*Thiat*
200	T4_5	KY594098	*Saccharomyces DGGE band*	183	100	*Thiat*
201	T5_4	KY594099	*Zygosaccharomyces bailii*	188	80	*Thiat*
202	T5_7	KY594100	*Aspergillus penicillioides*	203	96	*Thiat*

Table 49. Identification of yeasts and molds, based on BLAST comparison in Gen-Bank, of the bands obtained by PCR-DGGE gel using universal primers NL1/LS2

[1]Band Number	Closest relative species	Accession No.	[2]Similarity Identity (%)
Yeasts:			
M2_1, H1_1, D4_3, A2_1, C1_1 C3_1	*Saccharomycopsis fibuligera*	KY594214	100
T1_5, M5_10, M3_9,M3_10, D4_8, A3_4,A1_3, C1_7	*Saccharomyces cerevisiae*	KY594246	100
C1_6, C2_3, C4_2, D2_2, M4_5	*Wickerhamomyces anomalus*	KY594179	100
P4_1, M4_2, M2_2	*Saccharomycopsis malanga*	KY594073	100
K1_6, K1_7, K1_6	*Kluyveromyces marxianus*	KY59408	98
M4_8	*Pichia guilliermondii*	KY594234	98
A2_6, A2_7, M4_13	*Pichia kudriavzevii*	KY594151	100
M4_6	*Candida tropicalis*	KY594232	99
C3_3, D4_6	*Hyphopichia burtonii*	KY594197	99
C2_4	Non-culturable*Hyphopichia*	KY594187	98
M4_11	*Candida glabrata*	KY594236	98
Molds:			
P2_4, M3_4	*Rhizopus oryzae*	KY594221	99
D5_11, D5_12, D4_11	*Aspergillus penicillioides*	KY5941420	99
D2_7 C4_7, C2_1,H1_6	*Aspergillus oryzae*	KY594049	99

[1]Each number corresponds to the bands indicated in Fig 2, 3, 4 and 5. The 26S rRNA fragments from the DGGE bands were aligned with GenBank reference sequences (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).
[2]Only above 98% similarities of bands were shown.

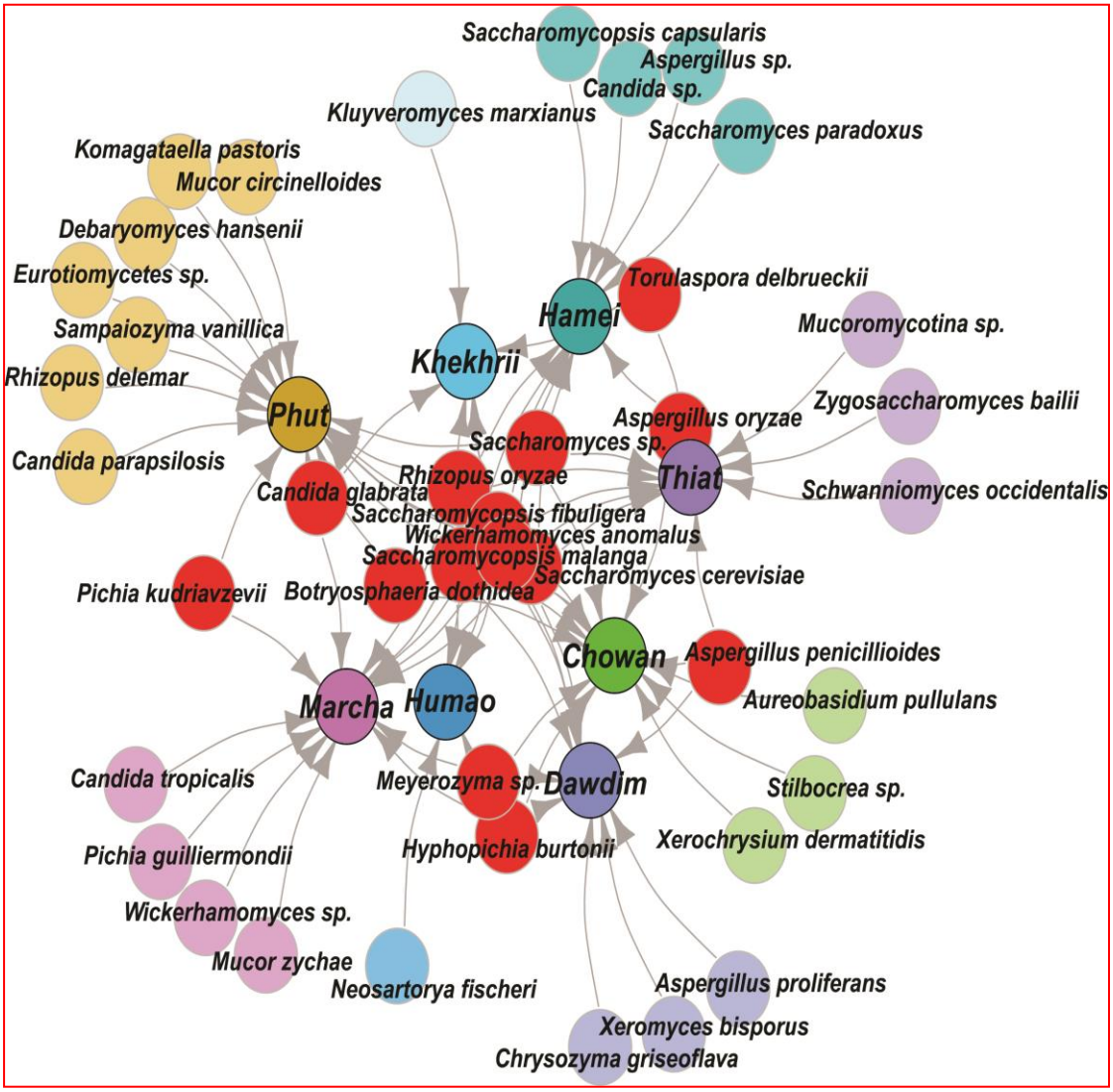


Figure 37. Graphical representation of all species identified in PCR-DGGE of 26SrRNA gene after sequencing. Shared species were represented in red color and sample specific unique species were represented in respective colors to the starter samples and arrow indicated the origin distribution patterns of these isolates.

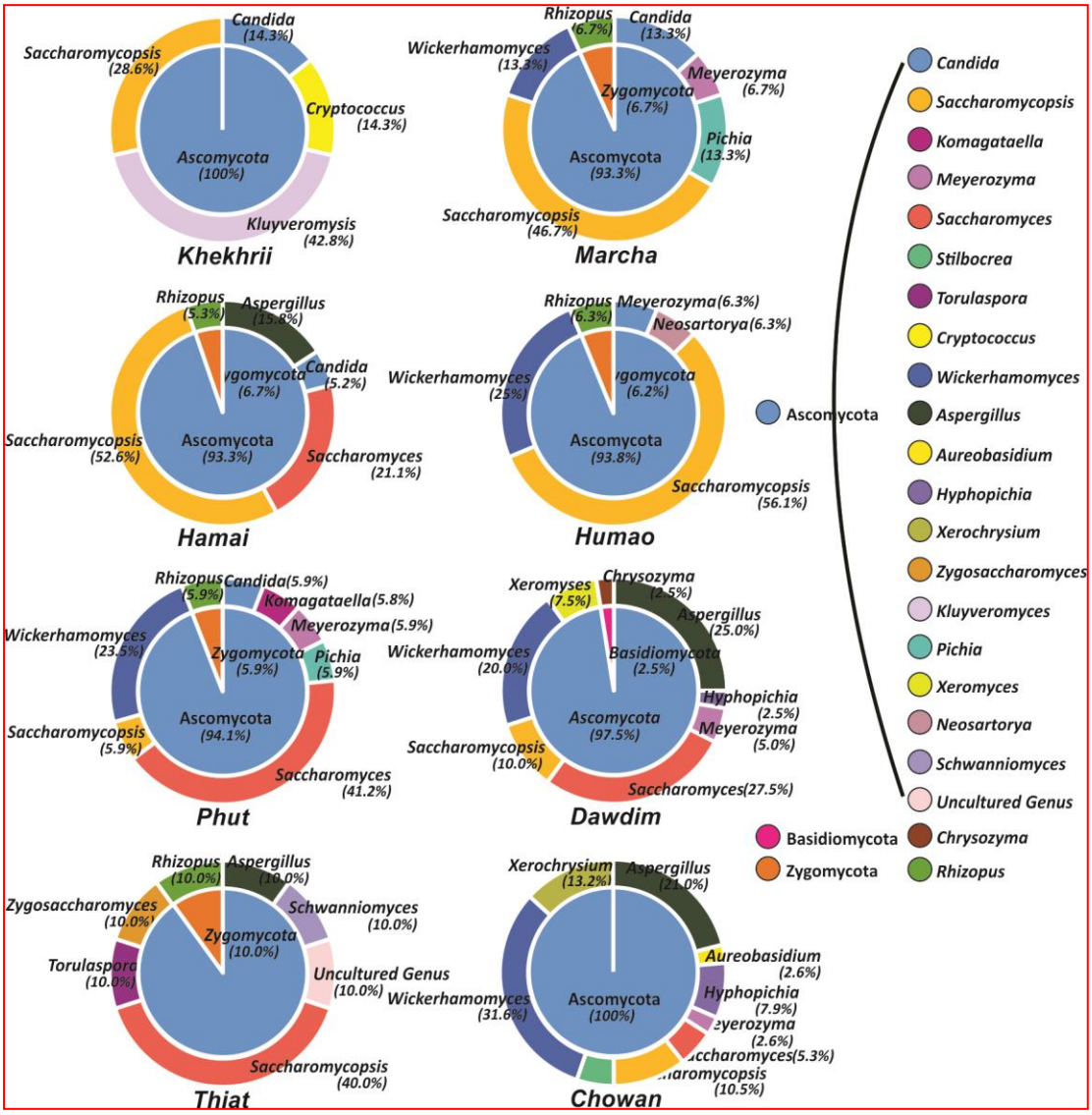


Figure 38. Genus and phylum level distribution of yeast and molds diversity in amylolytic starters. Genus and respective phylum presented here was based on 98 % identity cutoff value to the GeneBank database.

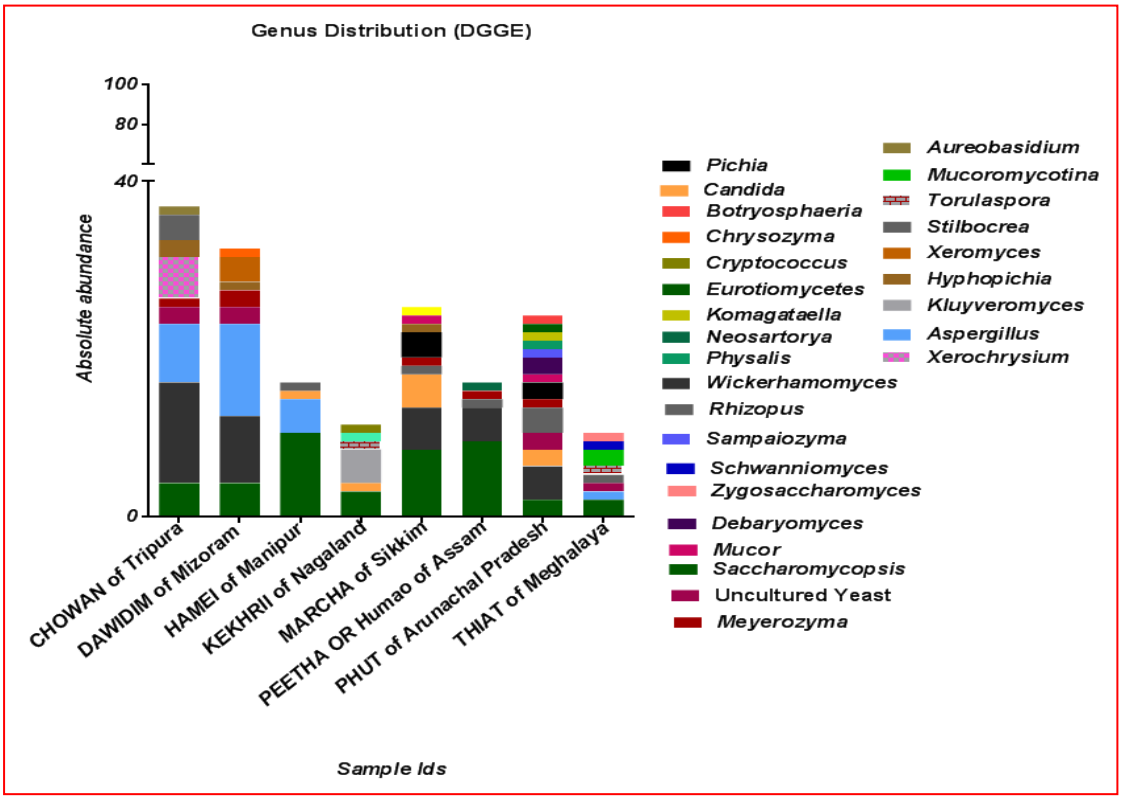


Figure 39. Complete genus level profile of yeast and molds in amylolytic starters analysed by PCR-DGGE.

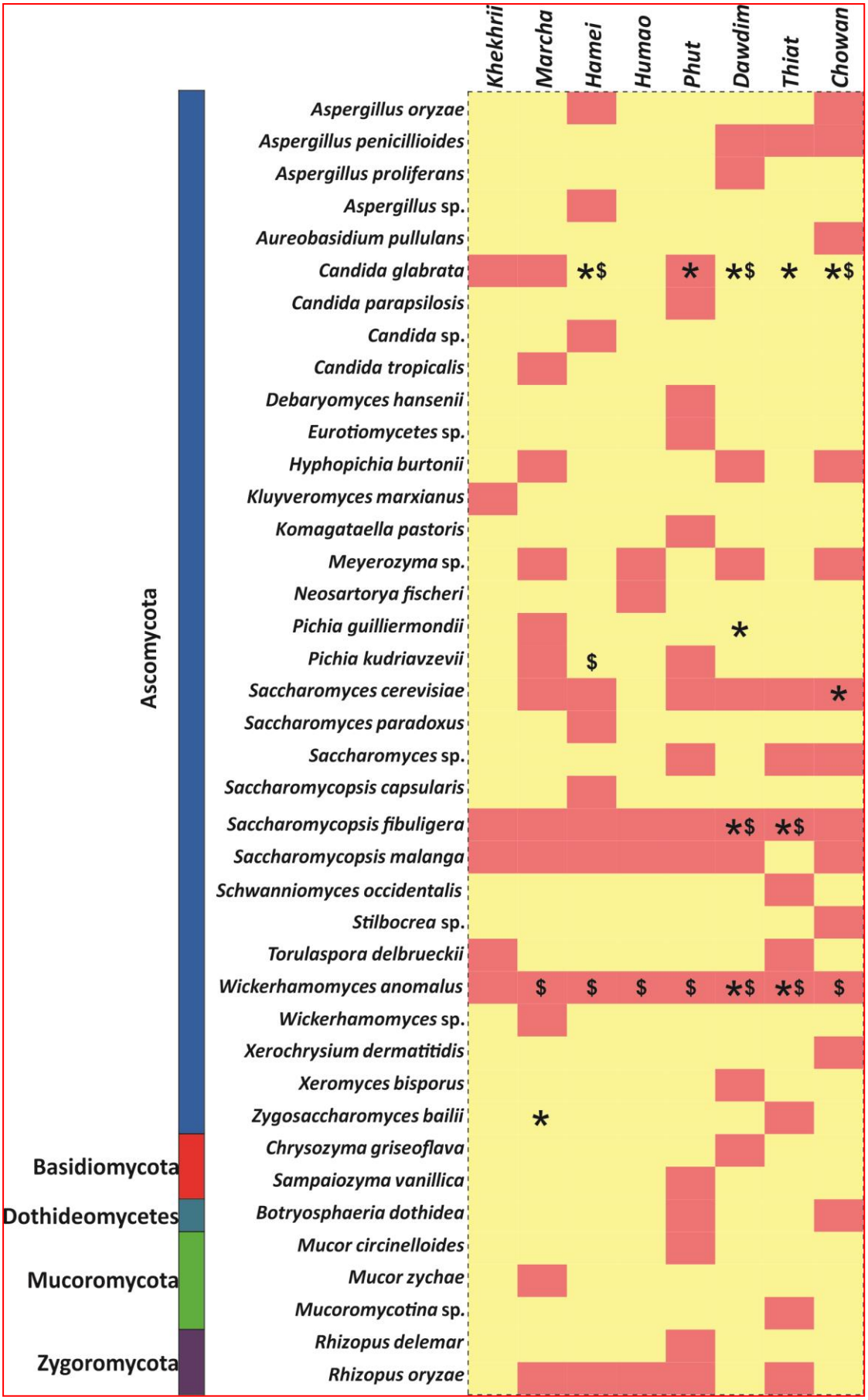

Khekhrii
Marcha
Hamei
Humao
Phut
Dawdim
Thiat
Chowan
Aspergillus oryzae
Aspergillus penicillioides
Aspergillus proliferans
Aspergillus sp.
Aureobasidium pullulans
Candida glabrata
Candida parapsilosis
Candida sp.
Candida tropicalis
Debaryomyces hansenii
Eurotiomycetes sp.
Hyphopichia burtonii
Kluyveromyces marxianus
Komagataella pastoris
Meyerozyma sp.
Neosartorya fischeri
Pichia guilliermondii
Pichia kudriavzevii
Saccharomyces cerevisiae
Saccharomyces paradoxus
Saccharomyces sp.
Saccharomycopsis capsularis
Saccharomycopsis fibuligera
Saccharomycopsis malanga
Schwanniomyces occidentalis
Stilbocrea sp.
Torulaspora delbrueckii
Wickerhamomyces anomalus
Wickerhamomyces sp.
Xerochrysium dermatitidis
Xeromyces bisporus
Zygosaccharomyces bailii
Chrysozyma griseoflava
Sampaiozyma vanillica
Botryosphaeria dothidea
Mucor circinelloides
Mucor zychae
Mucoromycotina sp.
Rhizopus delemar
Rhizopus oryzae
Ascomycota
Basidiomycota
Dothideomycetes
Mucoromycota
Zygoromycota

Figure 40. Heatmap showing the consensus species diversity observed during PCR-DGGE, Biolog identification hits and ITS-region gene sequencing of yeast isolates. We used presence- absence value of PCR-DGGE species data to generate heatmap whereas red color indicates the presence and in other hand yellow color represents absence value. Other datasets were mapped over the heatmap like: Biolog identification (*) and ITS-region gene sequencing of yeast isolates ($).

HIGH THROUGHPUT AMPLICON SEQUENCING RESULTS

We selected two samples of *marcha* of Sikkim and *thiat* of Meghalaya for Next Generation Sequencing (NGS) technique using High-throughput amplicon sequencing are traditionally prepared amylolytic starters use for preperation of different ethnic mild alcoholic beverages in Sikkim and Meghalaya states in India. In the present study we have tried to analyse the complete fungal population (mycobiome) composition of *marcha* and *thiat* by using high throughput sequencing (NGS). The raw sequence data of two amylolytic starters' *marcha* and *thiat* were analysed by QUIME software and quality trimming of ITS gene yielded ~0.29 million quality reads in both amylolytic starters *marcha* and *thiat*, respectively which was used for subsequent data analysis. The taxonomic assignment of sequences with the reference database resulted into 5,015 (OTUs) operational taxonomic units. The amplicon sequencing of ITS region was found to be 87.5% ± 17.6% (mean ± SD) indicating taht majority of the diversity was captured. The estimates of alpha diversity indices revealed significant differences between *thiat and marcha* when computed for yeast and molds diversity (Table. 50). From the analysed raw NGS data it was observed that fungal species richness depicts higher in *marcha* (5.25) over *thiat* (5.0). Significant variations were also observed in non-parametric Shannon index for yeast and mold population follow the reverse trend with *marcha* (2.25) and *thiat* (1.80). The sequencing raw data results showed the higher diversity of filamentous fungi (molds) in *thiat* whereas *marcha* showed the higher yeasts diversity. Yeats and mold ITS gene sequencing and taxonomic raw data analysis revealed the predominance of yeast phylum *Ascomycota* (98.6%) in starter *thiat*, whereas the distribution

of filamentous fungi phyla *Zygomycota* was only 1.4% (Figure. 41). However, in amylolytic starter *marcha* only yeast phylum *Ascomycota* constituted the 100 % of fungal diversity (Figure. 41). Filamentous fungi phylum was not detected in *marcha*. The class level distribution of *marcha was Saccharomycetales, sordariomycetes* and class level distribution of *thiat were Mucoromycotina, eurotiomycetes, dothideomycetes, Saccharomycetales* (Figure. 42). Distributions of fungi (filamentous fungi 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 average distributions of yeasts at the level of order/family in *marcha* were *Saccharomycetaceae* (37.5%), *Saccharomycetales* (50%) and *Amphisphaeriaceae* (12.5%) (Figure. 43). Distributions of yeasts genera in *marcha* were *Wickerhamomyces* (25%), *Candida* (25%), *Kazachstania* (25%), *Saccharomyces* (12.5%) and *Pestalotiopsis* (12.5%) (Figure. 45a). The filamentous mold genera distribution in *thiat* were *Aspergillus* (15.7%), *Aureobasidium* (3.9%) and *Mucor* (2.7%) and unidentified genera (77.7%) (Figure. 45a). The genera which were unidentified represented the yeast phylum *Ascomycota* in *thiat*. The sequence reads showed the species of filamentous fungi were *Aspergillus penicillioides, Mucor circinelloides* and *Aureobasidium pullulans*, whereas the yeasts species were *Wickerhamomyces anomalus, Kazachstania exigua* and *Candida quercitrus* (Figure. 45b). The sequence reads showed that sample *marcha* was yeast dominant while the starter sample *thia*t *mold* dominated (Figure. 46 a, b). This is the first report on fungal diversity of *marcha* and *thiat* traditionally prepared amylolytic starters of India using high throughput sequencing technique. The raw sequence data

analysis reveals that fungal composition dominated by *Ascomycota* as the dominant phylum and the presence of *Zygomycota* in starter *thiat* distinguishes it from the *marcha* (Figure. 41).

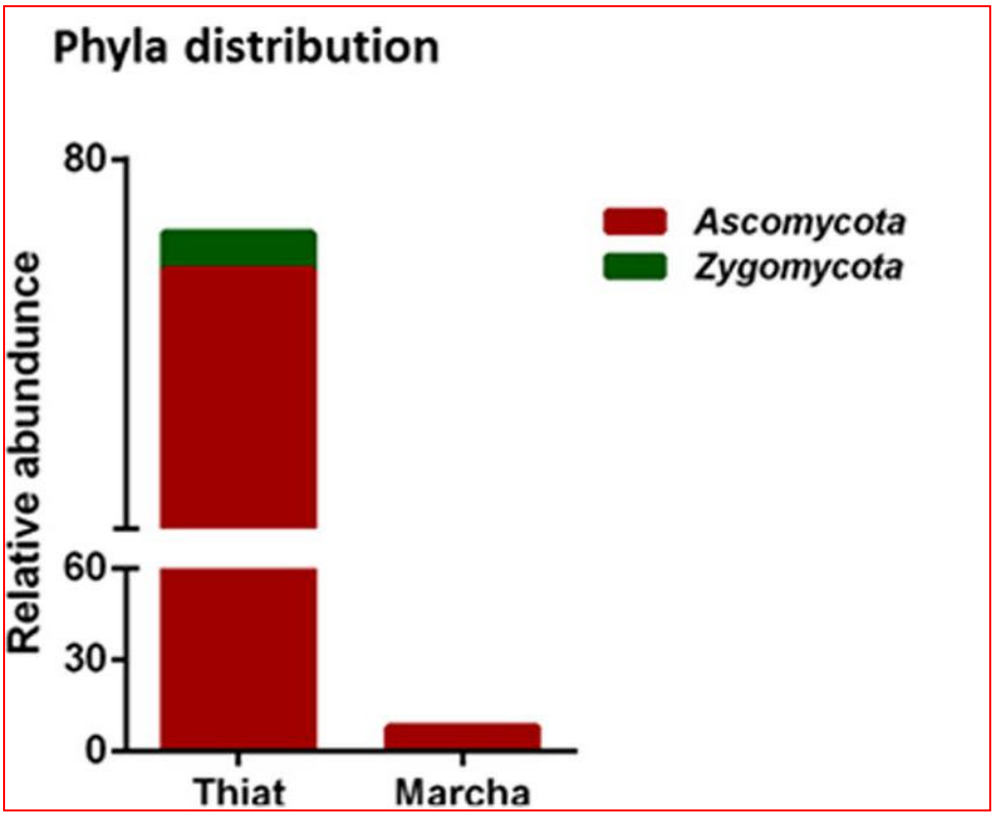


Figure 41. Fungal phylum composition in ethnic amylolytic starter cultures *marcha* and *thiat*, respectively.

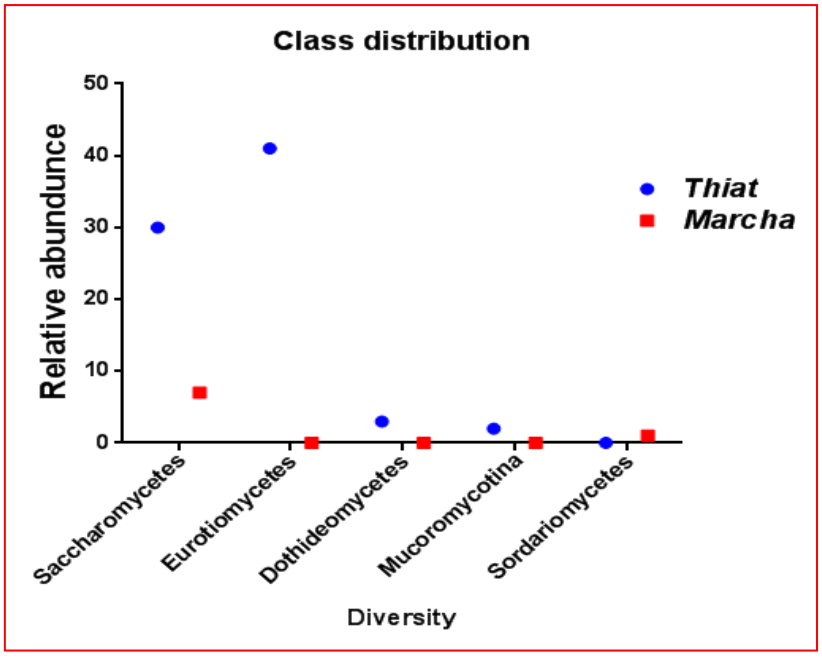


Figure 42. Fungal Class composition in ethnic amylolytic starter cultures *marcha* and *thiat*, respectively.

Figure 43. Order distribution of fungal composition in ethnic amylolytic starter cultures *marcha* and *thiat*, respectively.

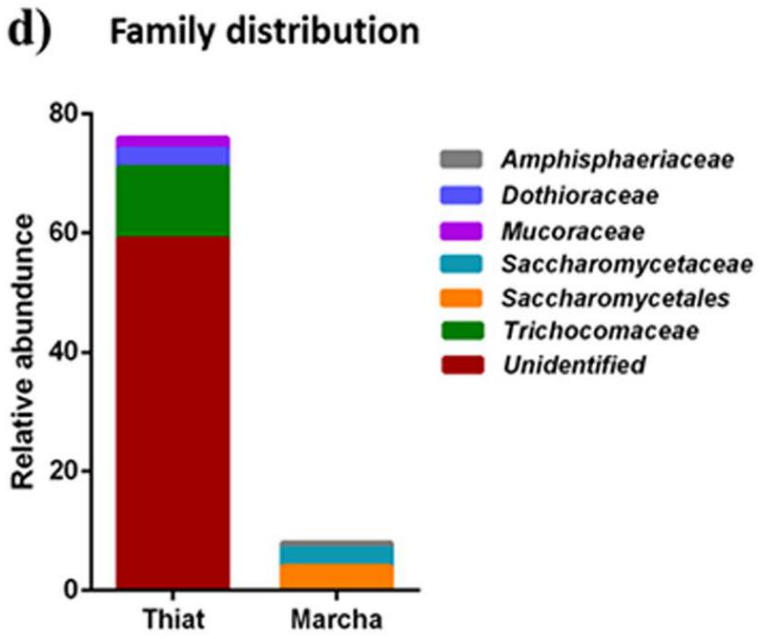


Figure 44. Family phylum composition in ethnic amylolytic starter cultures *marcha* and *thiat*, respectively.

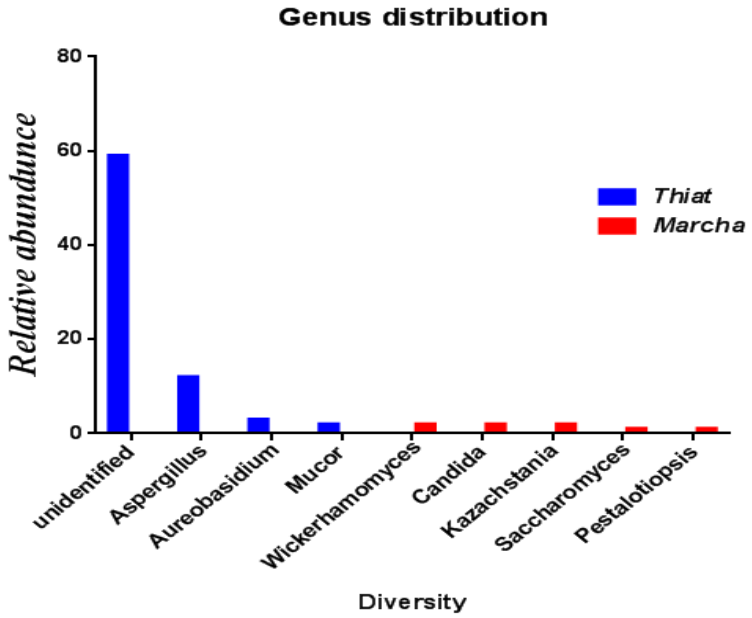


Figure 45a. Genus composition in ethnic amylolytic starter cultures *marcha* and *thiat*, respectively.

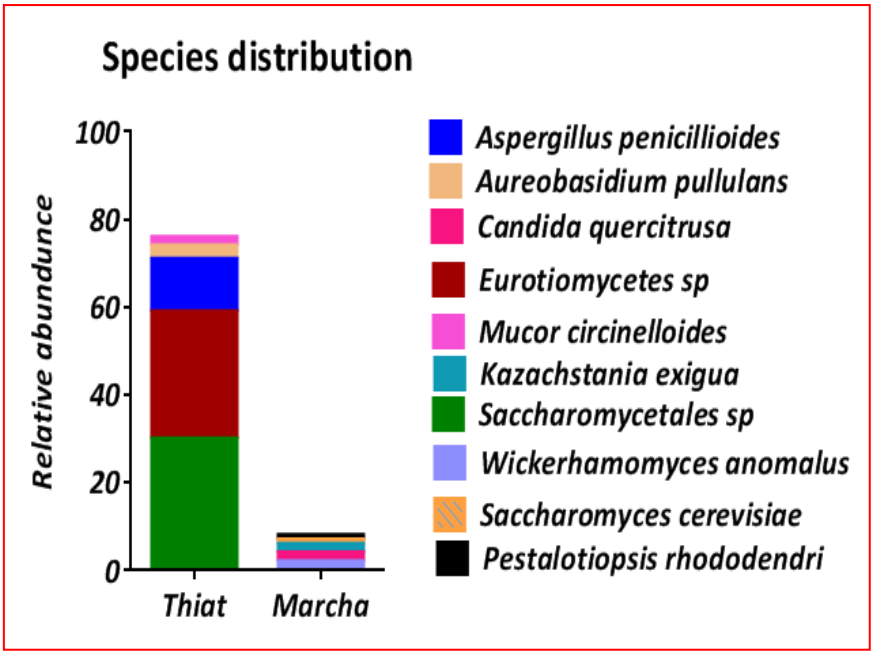


Figure 45b. Complete fungal speices composition in ethnic amylolytic starter cultures *marcha* and *thiat*, respectively.

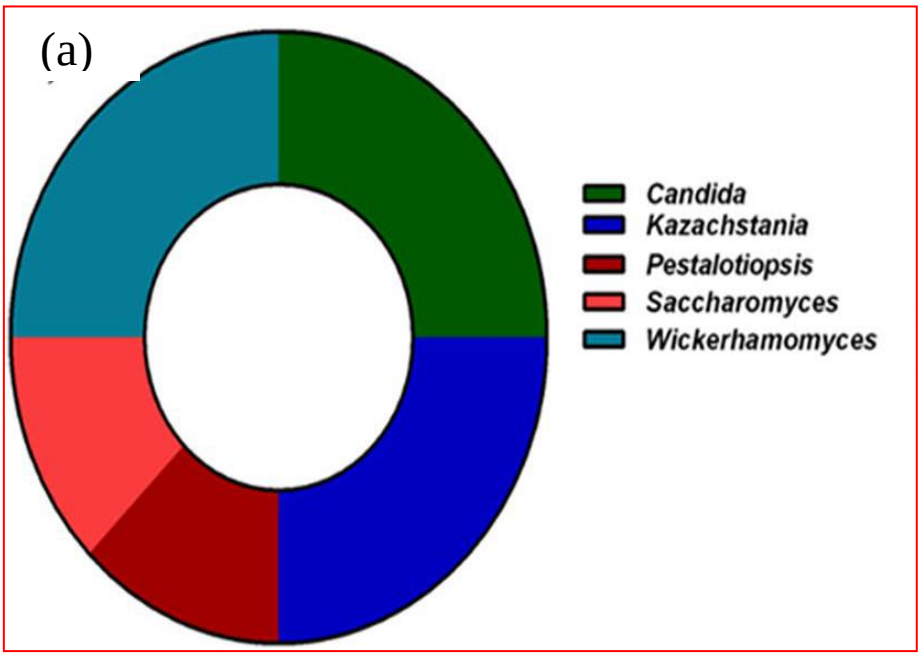


Figure 46(a). Yeast speices composition of ethnic amylolytic starter, *marcha*

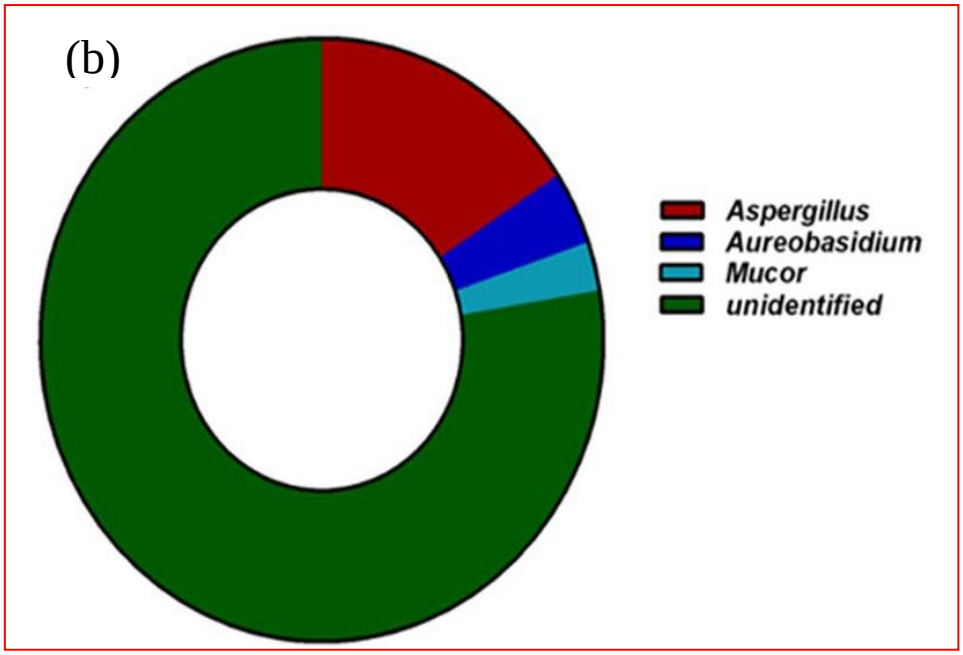


Figure 46(b). Mold speices composition of ethnic amylolytic starter culture, *thiat*

Table 50. Alpha diversity estimation of NGS sequencing data. Non parametric alpha diversity was calculated for ethnic amylolytic starter cultures *marcha* and *thiat*

Fungal- Alpha diversity

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

Alpha-amylase and glucoamylase activities of yeasts isolates from amylolytic starters

Fourty-six slecteed representative strains of yeasts isolated from different samples of amylolytic starters were tested for a-amylase and glucoamylase activity (Table. 51). All the four selected strains of the *Saccharomycopsis fibuligera* showed best a-amylase and glucoamylase activity. The a-amylase activity of *Saccharomycopsis fibuligera* ranged between (2.76-4.76)U/g. The activity glucoamylase activity of *Saccharomycopsis fibuligera* ranged between 2.27-3.30 U/g. The a-amylase activity of all 32 starins of *Wickerhamomyces anomalus* ranged between (2.20-4.44)U/g. The activity glucoamylase activity of *Wickerhamomyces anomalus* ranged between (2.20-3.33)U/g. The a-amylase activity of all four *Pichia anomala* ranged between (2.76-4.44)U/g. The activity glucoamylase activity of *Pichia anomala* ranged between 2.20-3.30 U/gm. The a-amylase activity of all three *Candida glabrata* ranged between (3.33-4.44)U/g. The activity glucoamylase activity of *Pichia anomala* ranged between 2.20-2.27U/g. The a-amylase activities of *Pichia kudriavzevii* strains ranged between (2.70-2.76)U/g. The activity glucoamylase activity of *Pichia kudriavzevii* strains ranged between (2.20-2.30)U/g. The a-amylase activity of one *Pichia terricola* was (2.00) U/gm and glucoamylase activity was (1.60)U/g. The highest a-amylase activity of *Saccharomycopsis fibuligera* ranged between (2.76-4.76)U/gm. The activity glucoamylase activity of *Saccharomycopsis fibuligera* ranged between (2.27-3.30)U/g. From the tabulated results it has been found that *S. fibuligera* and *Wickerhamomyces anomalus* yeast species showed best a-amylase and glucoamylase activities.

Table 51. Alpha-amylase and Gluco-amylase activities of identified yeasts isolated from Amylolytic starters of North East India

Sl. No	Name of Starter	Isolate code	Yeast (Genus/Species)	Alpha-amylase activity (U/gm)	Glucoamylase activity (U/gm)
1	*Marcha*	GM29	*Wickerhamomyces anomalus*	3.88±0.002	2.20±0.001
2	*Marcha*	GMY1	*Wickerhamomyces anomalus*	2.20±0.002	2.20±0.001
3	*Marcha*	GMY5	*Wickerhamomyces anomalus*	2.76±0.002	2.60±0.001
4	*Marcha*	GMY12	*Pichia anomala*	4.44±0.001	2.20±0.002
5	*Marcha*	GMY29	*Wickerhamomyces anomalus*	4.44±0.001	2.70±0.001
6	*Marcha*	GMY46	*Wickerhamomyces anomalus*	4.44±0.001	2.76±0.001
7	*Marcha*	MY15	*Wickerhamomyces anomalus*	3.33±0.004	2.70±0.004
8	*Thiat*	STY21	*Saccharomycopsis fibuligera*	3.33±0.004	2.32±0.001
9	*Thiat*	STY6	*Wickerhamomyces anomalus*	2.76±0.006	2.20±0.001
10	*Thiat*	STY24	*Pichia terricola*	2.20±0.008	1.60±0.001
11	*Thiat*	STY15	*Saccharomycopsis fibuligera*	4.44±0.004	2.27±0.001
12	*Thiat*	STY12	*Wickerhamomyces anomalus*	4.44±0.004	2.76±0.001
13	*Thiat*	STY3	*Wickerhamomyces anomalus*	4.96±0.003	2.30±0.001
14	*Thait*	STY49	*Wickerhamomyces anomalus*	4.96±0.002	2.76±0.001
15	*Chowan*	CHY28	*Candida glabrata*	3.88±0.004	2.27±0.001
16	*Chowan*	CHY39	*Wickerhamomyces anomalus*	3.33±0.002	2.20±0.001
17	*Chowan*	CX44	*Wickerhamomyces anomalus*	2.20±0.001	2.60±0.001
18	*Chowan*	CHX26	*Wickerhamomyces anomalus*	2.76±0.003	2.20±0.001
19	*Chowan*	CHX39	*Wickerhamomyces anomalus*	4.44±0.007	3.33±0.001
20	*Chowan*	CHY22	*Wickerhamomyces anomalus*	2.76±0.994	2.20±0.001
21	*Dawdim*	MY9	*Wickerhamomyces anomalus*	4.40±0.004	2.70±0.008
22	*Dawdim*	MY20	*Wickerhamomyces anomalus*	2.76±0.002	2.20±0.008
23	*Dawdim*	MY30	*Candida glabrata*	4.44±0.003	2.20±0.002
24	*Dawdim*	MY47	*Wickerhamomyces anomalus*	4.44±0.001	2.70±0.001
25	*Dawdim*	MY57	*Wickerhamomyces anomalus*	4.44±0.002	3.30±0.001
26	*Dawdim*	MY3	*Wickerhamomyces anomalus*	4.40±0.004	2.00±0.001
27	*Dawdim*	MY6	*Pichia anomala*	4.44±0.002	2.65±0.002
28	*Dawdim*	STY15	*Saccharomycopsis fibuligera*	2.76±0.002	2.27±0.001
29	*Dawdim*	XTY20	*Pichia anomala*	2.76±0.001	2.20±0.001
30	*Dawdim*	STY15	*Saccharomycopsis fibuligera*	4.76±0.002	2.27±0.001

31	*Kekhrii*	KY8	*Wickerhamomyces anomalus*	2.76±0.006	2.20±0.001
32	*Kekhrii*	KY20	*Wickerhamomyces anomalus*	2.76±0.001	2.20±0.001
33	*Khekhrii*	KY18	*Wickerhamomyces anomalus*	4.40±0.001	2.70±0.001
34	*Kekhrii*	KY27	*Pichia anomala*	4.44±0.001	3.30±0.007
35	*Kekhrii*	KY38	*Wickerhamomyces anomalus*	4.76±0.002	3.30±0.002
36	*Kekhrii*	KY45	*Wickerhamomyces anomalus*	4.44±0.005	3.33±0.008
37	*Hamei*	MY8	*Wickerhamomyces anomalus*	4.44±0.001	2.76±0.002
38	*Hamei*	HSY7	*Pichia kudriavzevii*	2.76±0.002	2.20±0.001
39	*Hamei*	AH45	*Candida glabrata*	3.33±0.006	2.20±0.001
40	*Hamei*	HSY7	*Pichia kudriavzevii*	2.70±0.002	2.30±0.001
41	*Huamo*	ASY3	*Wickerhamomyces anomalus*	2.76±0.001	2.20±0.002
42	*Humao*	ASY5	*Wickerhamomyces anomalus*	2.76±0.001	2.20±0.001
43	*Humao*	ASY5	*Wickerhamomyces anomalus*	2.76±0.001	2.20±0.003
44	*Humao*	ASY4	*Wickerhamomyces anomalus*	2.76±0.006	2.20±0.001
45	*Phut*	STY49	*Wickerhamomyces anomalus*	2.20±0.001	2.20±0.001
46	*Phut*	STY20	*Wickerhamomyces anomalus*	2.76±0.002	2.20±0.001

(N.B-One unit of a-amylase activity was defined as the amount of enzyme liberating 1 μmol of reducing sugars (glucose equivalents) per min at pH 7.0 at 40°C and was expressed as U/gm).

Ethanol Estimation

Screening of the all fourty six alcohol producing yeasts isolates from amylolytic starters was carried out and tabulated in (Table. 52). The alcohol production showed by *Saccharomycopsis fibuligera* in the ranged between (9.3-10.0%), *Wickerhamomyces anomalus* ranged between (8.80-11.60%), *Pichia anomala* ranged between (9.2-9.6%), *Candida glabrata* ranged between (9.80-10.50%), *Pichia kudriavzevii* ranged between (9.7-10.30%), *Pichia terricola* was (9.20%). Out of fourty six yeasts strains all showed the alcohol productions. *Wickerhamomyces anomalus* showed the highest alcohol production is about (11.60%) from *chwan* of Tripura and *Pichia anomala* from *khekhrii* showed the minimum production of alcohol about (8.3 %).

Table 52. Ethanol production by identified yeasts isolated from Amylolytic starters of North-East India

Sl. No	Name of Starter	Isolate code	Yeast (Genus/Species)	Ethanol production (%)
1	*Marcha*	GM29	*Wickerhamomyces anomalus*	9.8±0.002
2	*Marcha*	GMY1	*Wickerhamomyces anomalus*	9.2±0.003
3	*Marcha*	GMY5	*Wickerhamomyces anomalus*	9.1±0.002
4	*Marcha*	GMY12	*Pichia anomala*	9.2±0.004
5	*Marcha*	GMY29	*Wickerhamomyces anomalus*	9.1±0.001
6	*Marcha*	GMY46	*Wickerhamomyces anomalus*	8.7±0.001
7	*Marcha*	MY15	*Wickerhamomyces anomalus*	9.2±0.004
8	*Thiat*	STY21	*Saccharomycopsis fibuligera*	9.3±0.004
9	*Thiat*	STY6	*Wickerhamomyces anomalus*	9.6±0.006
10	*Thiat*	STY24	*Pichia terricola*	9.2±0.008
11	*Thiat*	STY15	*Saccharomycopsis fibuligera*	9.3±0.004
12	*Thiat*	STY12	*Wickerhamomyces anomalus*	8.2±0.004
13	*Thiat*	STY3	*Wickerhamomyces anomalus*	8.13±0.003
14	*Thait*	STY49	*Wickerhamomyces anomalus*	9.00±0.003
15	*Chowan*	CHY28	*Candida glabrata*	10.5±0.004
16	*Chowan*	CHY39	*Wickerhamomyces anomalus*	9.20±0.002
17	*Chowan*	CX44	*Wickerhamomyces anomalus*	9.50±0.004
18	*Chowan*	CHX26	*Wickerhamomyces anomalus*	11.60±0.003
19	*Chowan*	CHX39	*Wickerhamomyces anomalus*	9.30±0.007
20	*Chowan*	CHY22	*Wickerhamomyces anomalus*	9.10±0.994
21	*Dawdim*	MY9	*Wickerhamomyces anomalus*	9.20±0.004
22	*Dawdim*	MY20	*Wickerhamomyces anomalus*	8.70±0.002
23	*Dawdim*	MY30	*Candida glabrata*	9.80±0.003
24	*Dawdim*	MY47	*Wickerhamomyces anomalus*	9.40±0.001
25	*Dawdim*	MY57	*Wickerhamomyces anomalus*	10.80±0.002
26	*Dawdim*	MY3	*Wickerhamomyces anomalus*	9.5±0.004
27	*Dawdim*	MY6	*Pichia anomala*	9.6±0.002
28	*Dawdim*	STY15	*Saccharomycopsis fibuligera*	9.3±0.002
29	*Dawdim*	XTY20	*Pichia anomala*	9.0±0.003
30	*Dawdim*	STY15	*Saccharomycopsis fibuligera*	10.0 ±0.002
31	*Kekhrii*	KY8	*Wickerhamomyces anomalus*	10.0±0.006

32	*Kekhrii*	KY20	*Wickerhamomyces anomalus*	9.60±0.001
33	*Khekhrii*	KY18	*Wickerhamomyces anomalus*	9.80±0.002
34	*Kekhrii*	KY27	*Pichia anomala*	8.30±0.001
35	*Kekhrii*	KY38	*Wickerhamomyces anomalus*	9.60±0.003
36	*Kekhrii*	KY45	*Wickerhamomyces anomalus*	9.40±0.005
37	*Hamei*	MY8	*Wickerhamomyces anomalus*	9.40±0.001
38	*Hamei*	HSY7	*Pichia kudriavzevii*	9.70±0.003
39	*Hamei*	AH45	*Candida glabrata*	9.80±0.006
40	*Hamei*	HSY7	*Pichia kudriavzevii*	10.3±0.002
41	*Huamo*	ASY3	*Wickerhamomyces anomalus*	10.5±0.001
42	*Humao*	ASY5	*Wickerhamomyces anomalus*	11.2±0.001
43	*Humao*	ASY5	*Wickerhamomyces anomalus*	11.5±0.004
44	*Humao*	ASY4	*Wickerhamomyces anomalus*	10.5±0.006
45	*Phut*	STY49	*Wickerhamomyces anomalus*	10.0±0.005
46	*Phut*	STY20	*Wickerhamomyces anomalus*	10.00±0.003

DISCUSSION

INDIGENOUS KNOWLEDGE

In North East India, malting process for alcohol production is very rare or unknown, inspite, a traditionally prepared amylolytic (related to conversion of starch to sugar) starter (Hesseltine 1991; Tamang 2010c) in the form of dry, flattened, or round balls made up of rice/wheat for production of different traditional alcoholic beverages is common. Various types of non-food amylolytic statrers in the form of dry, solid, oval-flat cake-like starters viz. *marcha* of Sikkim, *humao* of Assam, *hamei* of Manipur, *thiat* of Meghalaya, *phut* of Arunachal Pradesh, *khekhrii* of Nagaland, *chowan* of Tripura and *dawdim* of Mizoram prepared by diverse groups of ethnic people of North East India for the production of mild alcoholic beverages were documented and studied. Amylolytic starters of North East India are quite similar to amylolytic starters cuotures of South East Asia such as *benh men/men*of Vietnam, *bubod* of the Philippines, *chiu/chu/daque* of China and Taiwan, *loogpang* of Thailand, *ragi* of Indonesia, *manna* and *manapu* of Nepal, *phab* of Bhutan and Tibet in China and *nuruk* in Korea (Steinkraus 1996; Tamang and Fleet 2006, Tamang 2016). Native skill of alcohol production by amylolytic starter culture technique is well recognized in the Himalayan regions of India, Nepal and Bhutan (Tamang 2010a). Amylolytic starter-making technology reflects the traditional method of 'sub-culturing'of desirable inocula from previous batch to new culture using rice as base substrates usig back-sloping technique (Hesseltine 1983; Steinkraus 1996; Tamang et al. 2016a). This technique preserves the essential mixed-microbiota in the dry form which retains its potency *in situ* for over a year or more for beverages production. During preparation of amylolytic starters actually the consortia of microorganisms are enumerated and preserved in rice or wheat base, source of starch as modern term medium, and use

glucose-rich wild herbs to supplement carbon source for growing microorganisms. These consortia of microorganism in the modern food science are called mixed-starter cultures (Tamang et al. 2016a).

Amylolytic starter culture is mostly prepared by the rural women belonging to different ethnic communities if North East India. Sometimes, rural men help women in collecting wild herbs and pounding them during preparation. This art of technology is protected as hereditary trade and passes from mother to daughters. It was documented that during preparation of amylolytic starters cultures, some locally available wild herbs and spices are added and makers believe that addition of wild herbs give more sweetness to the product and they also believe that addition of chillies and ginger during reparation is to get rid of devils that may spoil the product. This is actually to prevent growth of undesirable microorganisms that may inhibit the growth of native functional microorganisms in mixed starters (Soedarsono (1972). Hesseltine (1983) has speculated that the spices, which are known to be inhibitory to many bacteria and molds, are the agents that select the right population of microorganisms for fermentation.

Traditionally prepared amylolytic starters cultures are produced at home for commercial use in a few villages in North East India which have linkages to nearby local markets where starter-makers sell the products once or twice in a week. Earnings out of selling supplement the domestic expenses. These starter-making villages in North East may be considered as centres of microbial genetic resources or gene banks involved in preserving the native microbial diversity in foods. The Himalayan women have been sub-culturing and maintaining a consortium of functional microorganisms for alcohol production in the form of dry oval to flattened cake-like starter called *marcha* or *phab* for more than 2000 years (Tamang 2010a) The trade of traditional

starter-making is constantly increasing as unorganized industry sector and contributing the regional economy. However, there has been no affording of any government in North East India to declare it as a trade which we suggest that it should be recognized by Central or local governments as cottage-industry. These amylolytic starter-making villages in North East India may be preserved and the rural women involved may be encouraged to strengthen their knowledge of sub-culturing and preserving the necessary functional microorganisms and native skill more scientifically

Microbial isolation, enrichment in appropriate culture media, purification, characterizations based on the particular taxonomic keys, proper identification and proper nomenclature of microorganisms associated with fermented foods and beverages are important aspects of microbial systematic which ensures the quality control and normalised production of fermented foods (Tamang and Holzapfel 1999; Tamang 2012). Based on our documentation of indigenous or traditional skill and knowledge of different ethnic groups of people mostly rural women of North East India for preserving and 'sub-culturing' the microbiome, In this Thesis, we designed to profile the fungal community using both culture-dependent and culture-independent methods in different types of traditionally prepared amylolytic starters of North East India.

YEASTS DIVERSITY IN AMYLOLYTIC STARTERS

Production of alcoholic beverage and their antiques specified with the type of raw materials and types of starters used for fermentation is easily perceived now. Types of traditionally prepared amylolytic starter cultures may have different and distinct mycobiome species as a part of diversity associated with it; hence the differences may

underscore in geographic speciality (Jeyaram et al. 2011). Diversity of yeasts associated with amylolytic starters in Asia may be closely related to the raw materials used as well as the regional climate where they are produced (Lv et al. 2013). Forwarding with this hypothesis we examined yeasts diversity and community in different amylolytic starter cultures of North East India used in alcoholic beverage production as an ethnic constituent.

Phenotypic and Biolog Tests

An average population of 386 strains of yeasts isolated from 40 samples of amylolytic starters (*marcha, humao, hamei, thiat, phut, khekhrii, chowan* and *dawdim*) collected from North East states was10^5 to10^7cfu/g. The colony morphology of most of the yeasts cells were creamy white to cottony, smooth, regular, oval to circular, with bipolar to multi-polar budding patterns and the pseudo- to true mycelia. Tentatively the following yeast genera were phenotypically identified as *Saccharomyces* (6.0%), *Pichia* (15.0%), *Candida* (14.0%), *Issatchankia* (15.0%), *Kluveromyces* (11.0%), *Schizosaccharomyces* (13.0%), *Saccharomycopsis* (8.0%) and *Torulopsis* (18.0%). *Pichia* and *Candia* were the dominant yeasts. *Saccharomycopsis fibuligera, Saccharomycopsis capsularis, Pichia anomala, P. burtonii, Saccharomyces cerevisiae, S. bayanus* and *Candida glabrata*were isolated from *marcha* of Sikkim and Darjeeling hills earlier (Tamang and Sarkar 1995; Tsuyoshi et al. 2005). *Saccharomyce bayanus* and *Candida glabrata* are also reported from several other Asian amylolytic starters (Hadisepoetro et al. 1979, Hesseltine et al. 1988, Hesseltine and Kurtzman 1990, Deak 1991). Although the species of *Saccharomycesbayanus* have not been isolated from any other Asian amylolytic starters, the closely-related species of *Saccharomyces cerevisiae* was isolated from *ragi* of Indonesia and *banh*

men of Vietnam (Hesseltine et al. 1988; Lee and Fujio 1999). *Saccharomycopsis fibuligera* is the most dominant yeasts in *marcha* (Tamang and Sarkar 1995), which is typically found growing on cereal products (Hesseltine and Kurtzman 1990). The most frequent yeast species present in *hamei* was *Pichia anomala* (41.7 %), followed by *S. cerevisiae* (32.5 %) and *Trichosporon* sp. (8%), the identity of major groups was confirmed by additional restriction digestion of ITS region with Hind III, EcoRI, Dde I and Msp I (Jeyaram et al. 2008). Based on cultural morphological and biochemical identification studies mold strains as *Mucor* sp. and *Rhizopus oryzae* and yeasts *Candida tropicalis* and *Saccharomyces cerevisiae* were identified from *medombae,* amylolytic starter of Cambodia (Chim et al. 2015; Chay et al. 2017).

We identified 60 strains of yeasts based on Biolog test results wherein very few Biolog identified yeasts strains were showing ≥0.70% probability and ≥0.5 similarities index value. Results from Biolog system, where the profile of growth responses provides a metabolic fingerprint for each isolate (Prapahilong et al. 1997), showed more diversity of yeasts in amylolytic starters of North East India than phenotypic characterization based on probability and similarities index value. The Biolog results showed the reliability upto 99.9%, probably by introducing a number of co-metabolism tests and many assimilation tests and oxidation tests, which is not observed in conventional phenotypic identification systems (Kreger van Rij 1984). However, the Biolog system is often unreliable (Kellogg et al. 1998) and also it detects only microbes that are cultivable and able to grow in high-nutrient conditions contribute to substrate utilization (Stefanowiez 2006). Even with high reliability rates, both phenotypic and Biolog tests did not coincide with the molecular reference tests for the majority of isolates: when the identification results were compared to 18S

rRNA gene sequencing and species-specific PCR reactions (Nisiotou and Nychas 2007).

ITS-PCR

It has been previously reported that the ITS region gene analysis is a reliable routine technique for the differentiation of yeasts at species level (Clemente-Jimenez et al. 2004; Combina et al. 2005; Zott et al. 2008). Considering that species-specific PCR protocols target specific genes of genera and species, the reliability of ITS region gene sequences was considered to be 100% (Moraes et al. 2013). Another advantage of molecular culture-dependent method including ITS allows a collection of pure cultures that may be used for further selection of suitable yeast strains to improve quality of alcoholic beverages (Lv et al. 2013).

In this study, *Wickerhamomyces anomalus, Pichia anomala, Saccharomycopsis fibuligera* and *Candida glabrata* were identified in amylolytic starters of North East India using ITS analysis. The previous studies also reported *Candida glabrata, Pichia anomala* and *Saccharomycopsis fibuligera* from *marcha* of Sikkim based on 18S rDNA sequences (Tsuyoshi et al. 2005). The ITS-PCR analysis of *ragi*, amylolytic starter of Indonesia showed the dominance of *Pichia kudriavzevii* (Barus and Steffysia 2013). It has been reported that *Candida glabrata*, which is a moderate alcohol producer, has also been recovered in *kodo ko jaanr*, ethnic fermented finger millet beverage prepared by using *marcha* (Thapa and Tamang 2004) and *men*, Vietnamese amylolytic starter (Dung et al. 2007), indicating that it is involved in alcohol production. Non-*Saccharomyces* yeasts may contribute to flavor or aroma formation in the alcoholic beverage (Fleet 2003; Dung et al. 2006; Jolly et al. 2017). *Saccharomycopsis fibuligera, Saccharomyces cerevisiae, Wickerhamomyces anomala*, *Pichia* sp. and *Candida* sp. are the most common yeasts present in rice-

based starters of Asia (Lee and Fujio 1999; Xie et al. 2007; Jeyaram et al. 2008). Interestingly, *Wickerhamomyces anomalus*, probably the most abundant yeast, was reported for the first time from all the eight amylolytic starters of North East India using ITS-PCR method. The multiple sequence alignment of the ITS region gene sequences of *Wickerhamomyces anomalus* may be used for many purposes including inferring the presence of ancestral relationships between the sequences (Rampersad 2014). It may be noted that protein sequences that are structurally very similar can be evolutionarily distant which is referred to as distant homology (Li and Durbin 2010).

PCR-DGGE

Genomic DNA extracted directly from samples of amylolytic starters of North East India using the PCR-DGGE analysis showed diversity of yeasts *Wickerhamomyces anomalus, Saccharomyces cerevisiae, S. malanga,S. paradoxus, Saccharomycopsis fibuligera, Sm. Capsularis, Candida glabrata, C. tropicalis, Meyerozyma* sp., *Pichia guilliermondii*, and *P. kudriavzevi*. Some researchers have reported the microbial community in few traditionally prepared starters cultures and traditional alcoholic beverages using PCR-DGGE analysis such as principal amylase-producer yeast *Saccharomycopsis* (*Sm.*) *fibuligera* and ethanol-producers *Saccharomyces cerevisiae* in *banh men* of Vietnam (Thanh et al. 2008), *nuruk* of Korea (Jung et al. 2012), *yaa qu* and *hong qu* of China (Lv et al. 2012, 2013; Chen et al. 2014), respectively. *Sm. fibuligera* secretes considerable amount of α-amylase, glucoamylase, acid proteases and β-glucosidase, which are applied in the fermentation industry (Chi et al. 2009).The dominance of *S. cerevisiae* in *marcha, thiat, dawdim* and *phut* might be due to its competitive growth in the presence of fermentable sugars and its ethanol tolerance may be due to fast growth during various alcoholic fermentations (Dung et

al. 2006, 2007; Jeyaram et al. 2008). *S. cerevisiae* has also found to be one of the dominant yeasts in all amylolytic starters of North East India. *S. cerevisiae* is naturally dominant in alcoholic fermentations because of its competitive growth under strict anaerobic conditions and its tolerance to ethanol (Romano et al. 2006). *Wickerhamomyces anomalus,* a regular component in several types of Asia-Pacific alcohol fermentation starters (Limtong et al. 2002; Thanh et al. 2008), was detected in almost all eight amylolytic starter samples. *P. guilliermondii* was observed in *marcha* samples and also found in wheat-based *qu* for Chinese Shaoxing rice wine (Xie et al. 2007) and *hamei* of Manipur in India (Jeyaram et al. 2008), which can produce volatile phenols and esters in the initial stages of alcoholic fermentation (Moreira et al. 2005). *Pichia kudriavzevii, Wickerhamomyces anomalus, Sm. malanga, Kluyveromyces marxianus, Torulaspora delbrueckii, Hyphopichia burtonii, Sm. capsularis,* and *Debaryomyces hansenii* were also reported from other Asian starter cultures for the production of flavour and ethanol (Dung et al. 2006; Xie et al. 2007; Zhang et al. 2008; Thanh et al. 2008; Jung et al. 2012; Lv et al. 2013; Chen et al.. 2014). *Zygosaccharomyces bailii* is widely present in various food fermentations, such as wine, tea, and vinegar fermentations (Garavaglia et al. 2015), and also produced various flavor compounds including alcohol in Chinese *Maotai*-liquor (Xu et al. 2017).

In *chowan,* few pathogenic fungi were also detected such as *Xerochrysium dermatitidis,* which is a pathogenic fungus causing skin diseases (Pitt et al. 2013); and *Aureobasidium pullulans,* a ubiquitous black, yeast-like human fungal pathogen found in soil, water, air and limestone (Chan et al. 2011). These pathogenic fungi may be contaminated through various raw substrates including wild herbs, water, etc. during crude preparation of *chowan* by village people in Tripura. The presence of sub-

phylum: *Mucoromycotina*, which is the earliest mutualistic symbiosis fungus with *Haplomitriopsida* liverworts (Field et al. 2015), probably passed through the plants used during preparation of *thiat.*

Besides yeast community, some molds *Rhizpus* spp. and *Aspergillus* sp. were also detected by PCR-DGGE analysis in amylolytic starters except in *khekhrii* samples of Nagaland. Species of *Rhizopus* sp. and *Aspergillus* were reported from many Asian amylolytic starters (Yang et al. 2013; Zhu and Tramper 2013). The distributions of yeasts communities in amylolytic starters of North East India were higher in comparison to molds this may be due to low temperatures of that particular environment in North East India and also the substrates used for fermentation (Chi et al. 2009). These traditional amylolytic starters are the result of long term selection for preserving and cultivation the amylolytic and alcohol-producing native yeasts and fungi by ethnic people which has been practising the traditional process for centuries (Tamang 2010a; Londoño-Hernández et al. 2017). The PCR-DGGE analysis has some disadvantages due to its inability to determine the relative abundance of dominant species, differentiate between viable and nonviable cells, and difficulties in interpretation of multi-bands (Nam et al. 2011; Dolci et al. 2015). Besides, DNA extraction efficiencies vary between microorganisms since DGGE band intensity is not always correlated with population density (Ercolini 2004; Prakitchaiwattana et al. 2004; Lv et al. 2013).Selection of ethnic starters from different geographies for their mycobiome count gaining the importance of species diversity as indigenous property but size of the samples that may limits the true representation. Hence, counterpart suggesting the bigger pictures with larger size and wider the samples origins.

High-throughput Amplicon Sequencing Method

Our study revealed comprehensive fungal diversity analysis using high throughput amplicon sequencing approach of traditionally amylolytic starter from North East India. Quantitative differences were observed for the presence of fungal texa among the starters *marcha* and *thiat*; which could be the consequence of differences in the method of preparation, incubation period and most importantly the type of preservations. The Alpha diversity estimation of *marcha* and *thiat* using species richness and non-parametric Shannon index suggested less fungal diversity in *thiat* while *marcha* showed 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, less fungal diversity of *thiat* depicts the faster turnover from acidic to alkali with the presence of acid (Kosseva et al. 1998). Exploration of fungal diversity of traditionally prepared 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 previous report of culture-dependent studies showing the dominance of *Mucor* and *Rhizopus* genera of *Mucorales* in *marcha* (Tamang et al. 1998). Interestingly no filamentous molds were detected in *marcha* using the 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 filamentous fungi, limited sample size and/or age of the sample and finally also due to inadequate cell lysis which may not allowed the release of nucleases (Dolci et al. 2015). Our study was in accordance to the earlier 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 final

products (Nam et al. 2012). Thus, we assume that the factor of geographic environment including altitudes and climate play a more important role over the manufacturing process in resulting in the various microbial compositions of the amylolytic starter culture under study. Some other important factors that may affect the composition of fungal communities in amylolytic starters are level of hygiene, quality of the glutinous rice, water quality, 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* were also reported by PCR-DGGE method (Sha et al. 2016). ITS gene sequences analysis of *thiat* revealed the existence of *Aspergillus penicillioides, Aureobasidium pullulans* and *Mucor circinelloides* as the most dominant filamentous molds. At family level *Trichocomaceae, Dothioraceae* and *Mucoraceae* as are the major constituents of fungal community composition emphasizing the significant differences between *thiat* and *marcha* viz differences in starter substrates, preparation method, inoculums, consortia, geographical condition, hygiene, preservation technique, caloric values etc. In the present study *Ascomycota* was dominant in *marcha,*which was also reported based on NGS tools in Korean alcoholic beverages (Jung et al. 2012) and in Chinese liquors (Li et al. 2011). *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 (Li et al. 2013). Amylolytic starter culture-making technique preserves the consortia of microbial community which were co-existed in traditionally prepared amylolytic and alcohol producing starters (Tamang et al. 2016) and also preserves vast biological genetic resources, otherwise, which may be forced to disappear. Alcoholic beverages produced by using ethnic amylolytic starters in North East 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 (Tamang et al. 2010). Ethnic fermented beverages and mild alcoholic drinks have the potential to grow in to beverage industry if proper scientific and technical supports are applied to the existing indigenous practices of home based traditional alcoholic fermentation. Similarly, the fungal diversity of *xaj-pitha*, an amylolytic starter of Assam was investigated through a next generation sequencing approach involving Illumine platform based whole genome shotgun sequencing method and revealed the presence of amylase producers, such as *Rhizopus delemar, Mucor circinelloides, Aspergillus* sp., and ethanol producersyeasts*Candida glabrata, Debaryomyces hansenii, Wickerhamomyces ciferrii, Saccharomycescerevisiae, Meyerozyma guilliermondii* and *Dekkera bruxellensis* (Bora et al. 2016). The Next Generation Sequencing result of *nuruk,* an amylolytic starter of Korea represents simple and rapid method of studying microbial ecology that permits the analysis of hundreds of thousands of nucleotide sequences (Roh et al. 2010). The Phyla level distribution showed *Ascomycota* and *Zygomycota* phyla were dominant phyla in *marcha* and *thiat*. Similarly, *Ascomycota* and *Zygomycota* were the predominant phyla in Korean *nuruk*, constituting 85.4% (±31.1) and 14.3% (±30.9) of the fungal populations, respectively and *Basidiomycota* at a rate of 0.01% and yeast families were *Saccharomycopsidaceae, Trichocomaceae, Mucoraceae* and *Saccharomycetaceae* constituting 99.6% (Jung et al. 2012). *Aspergillus oryzae* isolated from Korean *nuruk* improves fermentation properties and rice wine quality (Yang et al. 2013). The fungal diversity in Korean traditional wheat-based starter *nuruk* by fungal ITS database revealed mycobiome composition of *Ascomycota* and *Zygomycota* (Bal et al. 2016). In this study, we also observed the dominance of *Ascomycota* and *Zygomycota* in amylolytic starters of North East India.

Enzymatic Activities and Alcohol Production

Amylases are of great significance in food fermentation and food industries for hydrolysis of starch (conversion of polysaccharides to monosaccharides) and other related oligosaccharides (Akpan et al. 1999; Pederson and Nielson. 2000). Our results revealed α-amylase and gluco-amylase activities of *Saccharomycopsis fibuligera* were higher than other identified yeasts in amylolytic starters of North East India. *Sm. fibuligera*, the most abundant yeast species, can secrete a large amount of a-amylase, glucoamylase, acid proteases, had been applied in the fermentation industry (Chi et al. 2009). From results it has been found that *Saccharomycopsis fibuligera* and *Wickerhamomyces anomalus* showed highest α-amylase and glucoamylase activities. It had been found that the yeast *Saccharomycopsis fibuligera* was the major amylolytic yeast in traditionally prepared amylolytic starters of Asia (Limtong et al. 2002; Thanh et al. 2008; Tsuyoshi et al. 2005). It was also reported that glucoamylase produced by *Sm. fibuligera* can digest native starch, which improves the breakdown of polysaccharides from the raw materials (pea and barley) of *daqu*, an amylolytic starter of China (Chi et al. 2009).

From our study it was found that out of 46 yeast strains all were alcohol-producers, some of them mild to strong. *Wickerhamomyces anomalus*showed the highest alcohol production is about (11.60%) from *chowan* of Tripura and *Pichia anomala* from *khekhrii* showed the minimum production of alcohol about (8.3 %). *Chowan* of Tripura with high alcohol producing yeasts may be a good choice from industrial point of view. *Wickerhamomyces anomalus*is the most common yeast in several types of Asian starters (Limtong et al. 2002; Sujaya et al.2001; Thanh et al. 2008; Tsuyoshi et al. 2005).*Saccharomyces cerevisiae* usually dominates in alcoholic

fermentations (Nout 2009; Urso et al. 2008) as it has the capability to grow under obligate anaerobic conditions (Li et al. 2011) was also observed in our study.

CONCLUSION

Food culture of North East India is unique due to vast ethnicity, diversity in agro-resources, geographical and climatic variations, which also symbolises the heritage, and socio-cultural aspects of a community of the regions.Practicing of "ethno-microbiology" by diverse groups of ethnic people of North East India for centuries has evolved the distinct dietary culture in the regions for production and management of the available food bio-resources which may be considered as the prime step of modern food technology, thereby supplementing the food ecosystem and enhancing the regional economy. The concept of 'ethno-microbiology' is important in the modern food microbiology since this traditional technology involves the process of conservation and crude sub-culturing of essential and functional microbiota or microbiome comprising consortia of both culturable and unculturable microorganisms using back-sloping method by the ethnic people for centuries.

We performed one of the successful trials to find out the mycobiome associated with eight different amylolytic starters of North East India analyzed by phenotypic and Biology tests, ITS-PCR, PCR-DGGE analysis and high-throughput amplicon sequencing techniques. Application of culture-independent methods has helped to profile the entire mycobiome community comprising both culturable and unculturable in traditionally prepared amylolytic starters of North east India. These results may enrich our knowledge of indigenous yeasts that may be present in the ethnic amylolytic starters and may be used to promote the development of unique

ethnic alcoholic beverages; moreover, data of amylolytic starters of North East India can be used as reference data base for the further research.

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.

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 starters in the form of dry, solid, oval-flat cake-like starters viz. *marcha* of Sikkim, *humao* of Assam, *hamei* of Manipur, *thiat* of Meghalaya, *phut* of Arunachal Pradesh, *khekhrii* of Nagaland, *chowan* of Tripura and *dawdim* of Mizoram; and to investigate the yeast communities by culture-dependent and culture-independent methods; and also to estimate the α-amylase and glucoamylase activities and alcohol productivity of the identified yeast strains. Starter-making technology reflects the traditional method of 'sub-culturing' of desirable inocula from previous batch to new culture using rice as base substrates by back-sloping, and are produced at home for commercial use in few villages where starter-makers sell the products for livelihood. The average population of yeast in all eight starters of North East India was 7.2 x 10^6 cfu/g. Ascertaining the cultured diversity, a total of 386 yeasts strains were isolated from 40 samples, characterized and tentatively identified yeast genera as *Saccharomyces, Pichia, Candida, Issatchenkia*, *Kluyveromyces, Schizosaccharomyces*, *Saccharomycopsis* and *Torulopsis*. The Biolog identification system was used for the identification of 60 representative yeasts out of 386 isolates from ethnic amylolytic starters of North East India. The distributions of yeasts *Pichia anomalus* (52%), *Candida glabrata* (12%), *Debromyces castelii* (4%), *Saccharomycopsis fibuligera* (4%), *Debromyces polymorphus* (2%), *Pichia terricola* (4%), *P. trelalophila* (2%), *P. onychis* (2%), *P. subpeliculum* (2%), *Phaphia rhodozymas* (2%), *Rhhodotorula acheniorium* (2%), *Rhodotolura bacarum* (2%), *Rhodotorula aurantaea* (2%), *Saccharomyces ceriviciae* (2%), and

Zygosaccharomyces bailii (2%) were observed.The phylum level distribution of yeast in amylolytic starter analysed by Biolog tests were Ascomycota, Basidiomycota, Zygomycota, Mucoromycotina, and Dothideomycetes. Biolog results showed maximum diversity in all samples.

Out of 386 isolates, 46 representatives of yeast strains were identified from eight amylolytic starters of North East India by using ITS-PCR molecular method. From the ITS-PCR results it was found that the average distributions of yeasts were *Wickerhamomyces anomalus* (47.4%), *Candida glabrata* (18.8%). *Pichia anomala* (13.4%), *Pichia kudriavzevii* (7.9%) *Saccharomycopsis fibuligera* (5.0%) and *Pichia terricola* (3.8%).It was observed that species richness (R) was higher in *dawdim*, *hamei*, and *thiat* samples than that of *marcha khekhrii*, *chowan* and *phut*. *Wickerhamomyces anomalus* was dominant in all starters. The highest Shanon index was observed in *dawdim* samples and lowest was observed in *phut*.

We applied culture-independent method by directly extracting genomic DNA from 40 samples of amylolytic starters using the PCR-DGGE analysis. We targeted D1 and D2 domain of 26S rRNA gene (large ribosomal subunit) of yeast and mold. Notably, the average distributions of yeasts in samples were *Saccharomyces cerevisiae* (16.5%), *Saccharomycopsis fibuligera* (15.3%), *Sm. Malanga* (11.7%), *Wickerhamomyces anomalus* (11.3%), *Kluyveromyces marxianus* (5.3%), *Meyerozyma* sp. (2.7%), *Candida glabrata* (2.7%), *Saccharomyces* sp. (1.3%), *Hyphopichiaburtonii*(1.2%), *Schwanniomycesoccidentalis* (1.1%), *Pichia kudriavzevi*(1.0%), *Torulaspora delbrueckii* (1.0%), *Zygosaccharomycesbailii* (1.0%), *Pichia guilliermondii* (1.0%), *Candida parapsilosis* (0.4%), *Komagataella pastoris* (0.3%), *Sacch.*

Capsularis (0.6%), *S. Paradoxus* (0.6%), and *C. tropicalis* (0.1%). Similarly, the average distributions of molds were *Aspergillus penicillioides* (5.0%), *Rhizopus oryzae* (3.3%), sub-phyllum: *Mucoromycotina* (2.1%), *Cryptococcus amylolentus* (1.7%), *Xerochrysium dermatitidis* (1.6%), *Aspergillusoryzae* (1.3%), *Neosartorya fischeri* (0.8%), *A. proliferans* (0.6%), *Chrysozyma griseoflava* (0.6%), *Stilbocrea* sp. (0.6%), *Mucor circinelloides* (0.5%), *Aureobasidium pullulans* (0.4%) and *Xeromyces bisporus* (0.3%). Interestingly, we observed the distinct species were more than the shared species.

We also studied samples of *marcha* of Sikkim and *thiat* of Meghalaya using one of the powerful culture-independent tools by Next Generation Sequencing (NGS) method represented by high-throughput amplicon sequencing approach to profile complete fungal diversity. The sequencing raw data showed the higher diversity of molds 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 phyla *Zygomycota*was only 1.4%. 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%). Whereas, the average distributions of yeasts at the level of order/family in *marcha* were *Saccharomycetaceae* (37.5%), *Saccharomycetales* (50%) and *Amphisphaeriaceae* (12.5%). Distributions of yeasts genera in *marcha* were *Wickerhamomyces* (25%), *Candida* (25%), *Kazachstania* (25%), *Saccharomyces* (12.5%) and *Pestalotiopsis* (12.5%).

Mold genera distributions in *thiat* were*Aspergillus*(15.7%), *Aureobasidium* (3.9%) and *Mucor* (2.7%) and unidentified genera (77.7%). The genera, which were unidentified, represented the yeast phylum *Ascomycota* in *thiat*. The sequence reads showed the species of molds were *Aspergillus penicillioides*, *Mucor circinelloides* and *Aureobasidium pullulans*, whereas the yeasts species were *Wickerhamomyces anomalus*, *Kazachstania exigua* and *Candida quercitrus*. The Alpha diversity estimation of *marcha* and *thiat* using species richness and non-parametric Shannon index suggested less fungal diversity in *thiat* while *marcha* showed the higher assemblage of fungal diversity with dominance of yeast phylum *Ascomycota*.

The enzymatic analysis of 46 yeasts strains showed the highest a-amylase activity by *Saccharomycopsis fibuligera* (4.76U/g) and glucoamylase activity by *Saccharomycopsis fibuligera* ranged between (3.3U/g).We also screened yeast strains for their alcohol-producing abilities. Alcohol production showed by *Wickerhamomyces anomalus* was (8.80-11.60%), *Saccharomycopsis fibuligera* (9.3-10.0%), *Pichiaanomala* (9.2-9.6%), *Candida glabrata* (9.80-10.50%), *Pichia kudriavzevii*(9.7-10.30%), *Pichia terricola* (9.20%). *Wickerhamomyces anomalus* isolated from *chowan* of Tripura showed the highest alcohol production.

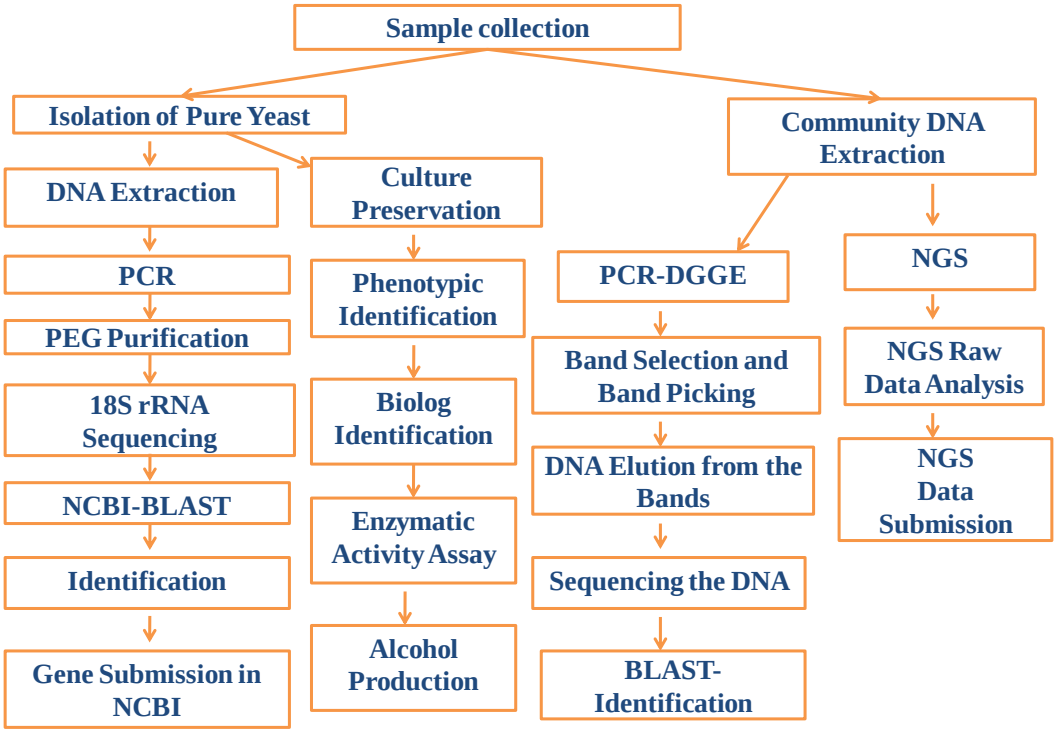
The present Thesis has provided the complete information on yeast and fungal communities of amylolytic starters of North East India, analysed by culture-dependent methods (phenotypic test, Biolog system, and ITS-PCR) and culture-independent methods (PCR-DGGE and NGS techniques by high-throughput amplicon sequencing) as well as their enzymatic activities and alcohol productivity.

This is the first report on complete profiles of mycobiome communities with vast diversity as well as their enzymatic and alcohol-producing abilities associated with traditionally prepared amylolytic starters of North East India: *marcha* of Sikkim, *humao* of Assam, *hamei* of Manipur, *thiat* of Meghalaya, *phut* of Arunachal Pradesh, *khekhrii* of Nagaland, *chowan* of Tripura and *dawdim* of Mizoram. Results generated in this Thesis may enrich information on composition of indigenous mycobiome that may be present in the ethnic amylolytic starters and may be used to promote the development of unique ethnic alcoholic beverages of North East India, moreover, data of amylolytic starters of North East India can be used as reference data base for the further research.

This Thesis has also documented the traditional practicing of "ethno-microbiology" by diverse groups of ethnic people of North East India which involves the process of conservation and crude sub-culturing of functional microbiome using back-sloping method. This is the worth documentation and recognition of the age-old wisdom and native skill of the ethnic people of North East India for alcohol production using amylolytic starters cultures.

Schematic representation of complete PhD work

during 2013-2018



Akpan, J.P. and Andre, T. (1999). The effect of a prior dissection simulation on middle school students' dissection performance and understanding of the anatomy and morphology of the frog. *Journal of Science Education and Technology* 8(2): 107-121. doi.org/10.1023/A:1018604932197.

Alegría, Á., González, R., Díaz, M. and Mayo, B. (2011). Assessment of microbial populations dynamics in a blue cheese by culturing and denaturing gradient gel electrophoresis. *Current Microbiology* 62: 888–893. doi:10.1007/s00284-010-9799-7.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*215: 403–410. doi:10.1016/S0022-2836(05)80360-2.

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.

AOAC, (2016). Official Method of Analysis, 20th Edition. Association of Official Analytical Chemists, Virginia.

Ardhana, M.M. and Fleet, G.H. (1989). The microbial ecology of tape *ketan* fermentation. *International Journal of Food Microbiology* 9(3): 157-165.

Astuli, M. (1999). History of development of *tempe*. In *The complete handbook of tempe*, (ed.) J. Agranoff, pp. 2-7. Singapore.

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.

Bal, J., Yun, S.H., Chun, J., Kim, B.T. and Kim, D.H. (2016). Taxonomic characterization, evaluation of toxigenicity, and saccharification capability of *Aspergillus* section Flavi isolates from Korean traditional wheat-based fermentation starter *nuruk*. *Mycobiology* 44(3): 155-161.

Barus, T. and Steffysia. (2013). Genetic diversity of yeasts from *Ragi tape* "starter for cassava and glutinous rice fermentation from Indonesia" Internal Transcribed Spacer (ITS) region. *Merit Research Journal of Food Science and Technology*. 1(3): 031-035.

Batra, L.R. (1986). Microbiology of some fermented cereals and grain legumes of India and vicinity. *Mycological Memoir (USA)*, 85-104.

Batra, L.R. and Millner, P.D. (1974). Some Asian fermented foods and beverages and associated fungi. *Mycologia* 66: 942-950.

Batra, L.R. and Millner, P.D. (1976). Asian fermented foods and beverages. *Developments in Industrial Microbiology* 17: 117–128.

Bhatia, A.K., Singh, R.P. and Atal, C.K. (1977). 'Chhang'the fermented beverage of Himalayan folk. *Indian food packer* 1-8.

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.

Bluhm, L. (1995). Distilled beverages. In: G. Reed (Eds.) Biotechnology vol. 5. food and feed production with microorganisms, Chemie: Weinheinverlag, 447-476.

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

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. *3Biotech* 6(2):153.doi:org/10.1007/s13205-016-0471-1

Caggia, C., Restuccia, C., Pulvirenti, A. and Giudici, P. (2001). Identification of *Pichia anomala* isolated from yogurt by RFLP of the ITS region. *International Journal of Food Microbiology* 71:71-73. doi:10.1016/S0168-1605(01)00556-6.

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 levûrechinoise. In *Annales de l'Institut Pasteur* 6: 604-620.

Campbell-Platt, G. (1987) *Fermented Foods of the World: A Dictionary and Guide*. Butterworths, England.

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.

Caputi, A., Ueda, M. and Brown, T. (1968). Spectrophotometric determination of ethanol in wine. *American Journal of Enology and Viticulture*19(3): 160-165.

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.

Chan, G.F.I., Puad, M.S., Chin, C.F. and Rashid, N.A. (2011). Emergence of *Aureobasidium pullulans* as human fungal pathogen and molecular assay for future medical diagnosis. *Folia Microbiol*ogy 56(5): 459-67.doi: 10.1007/s12223-011-0070-9.

Chay, C., Elegado, F.B., Dizon, E.I., Hurtada, W.A., Norng, C. and Raymundo, L.C. (2017). Isolation and identification of mold and yeast in *medombae*, a rice wine starter culture from Kompong Cham Province, Cambodia. *International Food Research Journal* 1(6): 708-717.

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. doi:10.1016/j.ijfoodmicro.2014.03.011.

Chi, Z.M., Chi, Z., Liu, G., Wang, F., Ju, L. and Zhang, T. (2009). *Saccharomycopsis fibuligera* and its applications in biotechnology. *Biotechnology Advances* 27(4): 423-431. doi: 10.1016/j.biotechadv.2009.03.003.

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.

Clemente-Jimenez, J.M., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Las Heras-Vázquez, F.J. and Rodríguez-Vico, F. (2004). Molecular characterization and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. *Food Microbiology* 21: 149–155. doi:10.1016/S0740-0020(03)00063-7.

Cocolin, L., Alessandria, V., Dolci, P., Gorra. And antsiou. (2013). Culture independent methods to assess the diversity and dynamics of microbiota during food fermentation. *International Journalof Food Microbiology* 167: 29–43.

Cocolin, L., Bisson, L.F. and Mills, D.A. (2000). Direct profiling of the yeast dynamics in wine fermentations. *FEMS Microbiology Letter* 189(1): 81-87.

Cocolin, L., Manzano, M., Aggio, D., Cantoni, C. and Comi, G. (2001). A novel polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) for the identification of *Micrococcaceae* strains involved in meat fermentations: its application to naturally fermented Italian sausages. *Meat Science* 58: 59-64.doi:org/10.1016/S0309-1740 (00)00131-5.

Combina, M., Mercado, L., Borgo, P., Elia, A., Jofré, V. and Ganga, A. (2005). Yeasts associated to Malbec grape berries from Mendoza, Argentina. *International Journal of Food Microbiology* 98: 1055-1061. doi:10.1111/j.1365-2672.2005.02540.x.

Cronk, T.C., Steinkraus, K.H., Hackler, L.R. and Mattick, L.R. (1977). Indonesian *tape ketan* fermentation. *Applied and environmental microbiology*33(5): 1067-1073.

Csardi, G. and Nepusz, T. (2006). The igraph software package for complex network research. *International Journal of Complex Systems* 1695(5): 1-9.

Darby, W.J. (1979). The nutrient contributions of fermented beverages. In: Gastineau, C.F., Darby, W.J. and Turner, T.B. (Eds.) Fermented Food Beverage In Nutrition, Academic Press, New York, 61-79.

Das, A.J., Deka, S.C. and Miyaji, T. (2012). 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: 101–107.

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.doi:org/10.2323/jgam.2016.11.004.

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

Dhamcharee, B. (1982). Traditional fermented food in Thailand. In:Saono, S., Winarno, W.J. and Karjarki, D. (Eds)*Traditional Food Fermentation as Industrial Resources in ASCA Countries,* The Indonesian Institute of Science (LIPI), Jakarta, 85-90.

Dobson, A., Cotter, P.D., Ross, R.P. and Hill, C. (2011). Bacteriocin production as a probiotic trait? *Applied and Environmental Microbiology* 78(1): 1-6.

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

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. doi:10.1016/j.fm.2005.05.002.

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 Science and Technology* 40(1): 130-135. doi:10.1016/J.LWT.2005.08.004.

Dwidjoseputro, D. and Wolf, F.T. (1970). Microbiological studies of Indonesian fermented foodstuffs. *Mycopathologia et mycologiaapplicata*41(3-4): 211-222.

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

El Sheikha, A.F., Condur, A., Métayer, I., Le Nguyen, D.D., Loiseau, G. and Montet, D. (2009). Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: an application to *Physalis* fruits from Egypt. *Yeast* 26(10): 567-573. doi:10.1002/yea.1707.

Elegado, F.B. and Fujio, Y. (1993). Polygalacturonase production by *Rhizopus* spp. *Journal of General and Applied Microbiology* 39: 409-418.

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, Springer, New Delhi, 323-340.

Ercolini, D. (2004). PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *Journal of Microbiological Methods* 56: 297-314. doi:10.1016/J.MIMET.2003.11.006.

Eren, A.M., Esen, O.C., Quince, C., Vineis, J.H., Morrison, H.G., Sogin, M.L. and Delmont, T.O. (2015). Anvi'o: an advanced analysis and visualization platform for 'omics data. *Peer J* 3: e1319.

Esteve-Zarzoso, B., Belloch, C., Uruburu, F. and Querol, A. (1999). Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. *International Journal of Systematic and Evolutionary Microbiology* 49: 329-337. doi:10.1099/00207713-49-1-329.

Field, K.J., Rimington, W.R., Bidartondo, M.I., Allinson, K.E., Beerling, D.J., Cameron, D.D., Duckett, J.G., Leake, J.R. and Pressel, S. (2015). First evidence of mutualism between ancient plant lineages (Haplomitriopsida liverworts) and *Mucoromycotina* fungi and its response to simulated Palaeozoic changes in atmospheric CO_2. *New Phytol.* 205(2): 743–756. doi: 10.1111/nph.13024.

Fleet, G.H. (1998). The microbiology of alcoholic beverages. In: B.J.B. Wood (Eds.) Microbiology of Fermented Foods, vol. I, London, U.K.: Blackie Academic and Professional. 217–262.

Fleet, G.H. (2003). Yeast interactions and wine flavour. *International Journal of Food Microbiology* 86: 11–22. doi:10.1016/S0168-1605(03)00245-9.

Garavaglia, J., Schneider, Rde. C., Camargo Mendes, S.D., Welke, J.E., Zini, C.A. and Caramão, E.B. (2015). Evaluation of *Zygosaccharomyces bailii* BCV 08 as a co-starter in wine fermentation for the improvement of ethyl esters

production. *Microbiology Research* 173: 59–65.doi:10.1016/j.micres.2015.02.002.

Garvie, E.I. (1960). The genus *Leuconostoc* and its nomenclature. *Journal of Dairy Research* 27(2): 283-292

Ghosh, C. and Das, A.P. (2004). Preparation of rice beer by the tribal inhabitants of tea gardens in Terai of West Bengal. *Journal of Traditional Knowledge* 3(4): 73-82.

Gordon, R.E., Haynes, W.C. and Pang, C.H.N. (1973). The Genus Bacillus (Agriculture Handbook no. 427). *Washington, DC: Agricultural Research Service, United States Department of Agriculture*. Washington D.C.

Greppi, A., Rantisou, K., Padonou, W., Hounhouigan, J., Jespersen, L., Jakobsen, M. and Cocolin, L. (2013). Yeast dynamics during spontaneous fermentation of mawè and tchoukoutou, two traditional products from Benin. *International journal of food microbiology*165(2): 200-207.

Grizard, S., Dini-Andreote, F., Tieleman, B.I. and Salles, J.F. (2014). Dynamics of bacterial and fungal communities associated with eggshells during incubation. *Ecology* and*Evlution*4: 1140–1157. doi:10.1002/ece3.1011.

Haard, N.F., Odunfa, S., Lee, C., Quintero-Ramírez, R., Lorence-Quiñones, A. and Wacher-Rodarte, C. (1999). Cereals: Rationale for fermentation. *Fermented cereals: A global perspective* 1: 1-27.

Hadisepoetro, E.S.S., Takada, N. and Oshima, Y. (1979) Microflora in ragi and usar. *Journal of Fermentation Technology* 57: 251-259.

Hesseltine, C.W. (1983). Microbiology of oriental fermented foods. *Annual Review of Microbiology* 37: 575-601.

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 Instituto de BiologíaserieBotánica* 60(1): 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*. *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. doi:10.1002/jsfa.2740440410.

Hirasawa, T., Yamada, K., Nagahisa, K., Dinh, T. N., Furusawa, C., Katakura, Y. and Shimizu, H. (2009). Proteomic analysis of responses to osmotic stress in laboratory and sake-brewing strains of Saccharomyces cerevisiae. *Process Biochemistry* 44(6): 647-653.

Hirooka, K., Yamamoto, Y., Tsutsui, N. and Tanaka, T. (2005). Improved production of isoamyl acetate by a sake yeast mutant resistant to an isoprenoid analog and its dependence on alcohol acetyltransferase activity, but not on isoamyl alcohol production. *Journal of bioscience and bioengineering* 99(2): 125-129.

Huang, H.T. (2000). Science and civilisation in China. Volume 6: Biology and biological technology. Part V: Fermentations and food science. *by J. Needham, Cambridge University Press, Cambridge*.

Hutchinson, C.M. and Ram-Ayyar, C.S. (1925). Bakhar the Indian rice beer ferment. *Memoirs of the Department of Agriculture in India, Bacteriology, Series* 1:137-168.

Iizuka, H. (1979). Art of mold in wine making in the Orient. *Shokuno–kagaku* 47: 30-38.

Inoue, T., Tanaka, J. and Mitsui, S. (1992) *Recent Advances in Japanese Brewing Technology* 2 (1): Gordon and Breach Science Publishers, Tokyo.

Iwen, P.C., Hinrichs, S.H. and Rupp, M.E. (2002). Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. *Medical Mycology* 40(1): 87-109.

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.

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., Singh, W.M., 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. doi:10.1016/j.ijfoodmicro.2008.02.029.

Jeyaram, K., Tamang. J.P., Capece, A. and Patrizia, R. 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: 569-78.

Jin, Z., Shimbo, T., Hosoe, Y. and Oyabu, T. (2005). Breath odor characteristics after drinking and identification of sake quantity. *Sensors and Actuators B: Chemical*, 108(1-2): 265-270.

Jolly, N.P, Augustyn O.P.H. and Pretorius I.S. (2006). The role and use of non-*Saccharomyces* yeasts in wine production. *South African Journal of Enology and Viticulture* 27: 15-39. doi:10.21548/27-1-1475.

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.doi:10.1016/j.fm.2011.09.008.

Kanlayakrit, W. and Booranasawettatham, S. (2004). *Screening of enzyme and alcohol producing microorganisms from Thai traditional fermentation*

starter (Lookpang) for Sato industry. In: Abstracts of the 42nd Kasetsart University Annual Conference, Bangkok, 3–6 Feb 2004.

Kanlayakrit, W. and Booranasawettatham, S. (2005). Identification of yeasts and molds isolated from Thai traditional fermentation starter (Lookpang) for Sato industry. In *43rd Kasetsart University Annual Conference, Bangkok*, 1-4.

Kanlayakrit, W., Nakahara, K., Teramoto, Y. and Hayashida, S. (1989). Raw starch-digesting glucoamylase from *Amylomyces* sp. 4-2 isolated from *Loogpang Kaomag* in Thailand. *Journal of the Faculty of Agriculture Kyushu University, 33*: 177–187.

Kato, K., Harada, T., Kuswanto, K. and Banno, I. (1976). Identification of *Endomycopsis fibuligera* isolated from *ragi* in Indonesia and properties of its crystalline glucoamylase. *Journal of Fermentation Technology* 54: 831–837.

Kellogg, J.A, Bankert, D.A and Chaturvedi, V. (1998). Limitations of the current microbial identification system for identification of clinical yeast isolates. *Journal of Clinical Microbiology* 36:1197-1200. doi:0095-1137/98/$04.0010

Kim, C.J. (1968). Microbiological and enzymological studies on *Takju* brewing. *Applied Biological Chemistry* 10: 69-100.

Kim, H.Y. (2013). Statistical notes for clinical researchers: assessing normal distribution using skewness and kurtosis. *Restorative dentistry & endodontics* 38(1): 52-54.

Kitamura, Y., Kusumoto, K.I., Oguma, T., Nagai, T., Furukawa, S., Suzuki, C. and Tamaki, H. (2016). Ethnic Fermented Foods and Alcoholic Beverages of Japan. In *Ethnic Fermented Foods and Alcoholic Beverages of Asia*, Springer, New Delhi, 193-236.

Kobayashi, Y., Tubaki, K. and Soneda, M. (1961). Several moulds and a yeast used for brewing native beer (*KodokoJar*) among the Sikkimese of India. *Journal of Japanese Botany* 36: 321-331.

Kodama, K. (1993). Sake-brewing yeasts. In *The yeasts*. Yeast technology, eds. A.H. Rose and J.SHarriso, London, U.K .Academic Press, 5:129-168.

Kodama, K. and Yoshizawa, K. (1977) Saké. In: Rose, A.H. (Eds.) *Economic Microbiology* vol. 1, New York, Academic Press, 432-475.

Korabecna, M. (2007). The variability in the fungal ribosomal DNA (ITS1, ITS2, and 5.8 SrRNA gene): its biological meaning and application in medical mycology. *Communicating Current Research Education Top Trend Application Microbiology* 2: 783-787.

Kosseva, M., Beschkov, V., Kennedy, J.F. and Lloyd, L.L. (1998). Malolactic fermentation in Chardonnay wine by immobilised *Lactobacillus casei* cells. *Process Biochemistry* 33(8):793797.doi.org/10.1016/S00329592(98)00049-1.

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.

Kozaki, M. and Uchimura, T. (1990). Microorganisms in Chinese starter '*bubod*' and rice wine '*tapuy*' in the Philippines. *Journal of Brewing Society of Japan* 85 (11): 818-824.

Kreger-van Rij, N.J.W. (1984). *The Yeasts, A Taxonomic Study*. Elsevier Science Publishers, Amsterdam

Krusong, W. (2014). Starter cultures. In J. D. Owens (Ed.), *Indigenous fermented foods of Southeast Asia*. Florida: CRC Press.

Kumar, V. and Rao, R.R. (2007). Some interesting indigenous beverages among the tribals of Central India. *Journal of Traditional Knowledge* 6(1): 141-143.

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(6): e0129864 doi:10.1371/journal. pone.0129864.

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.

Kurtzman, C.P., Fell, J.W. and Boekhout, T. (2011). *The Yeasts: A Taxonomic Study*, 5th edition, Elsevier, London.

Lappe-Oliveras, P., Moreno-Terrazas, R., Arrizón-Gaviño, J., Herrera-Suárez, T., García-Mendoza, A., & 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.

Las, Heras-Vazquez., Javier, F., Mingorance-Cazorla, L., Clemente-Jimenez, J.M. and Rodriguez-V.F. (2003). Identification of yeast species from orange fruit and juice by RFLP and sequence analysis of the 5.8S rRNA gene and the two internal transcribed spacers. *FEMS Yeast Research* 3(1): 3-9.

Lee, A.C. and Fujio, Y. (1999). Microflora of banh men, a fermentation starter from Vietnam. *World Journal of Microbiology and Biotechnology* 15: 57-62. doi:10.1023/A:1008897909680.

Lee, C.H. (1995). An introduction to Korean food culture. *Koreaa and Korean American studies Bulletin* 6(1): 6-10.

Lee, C.H. and Kim, M.L. (2016). History of fermented foods in Northeast Asia. In: Tamang, J.P. (Eds.) *Ethnic Fermented Foods and Alcoholic Beverages of Asia*, Springer, New Delhi. ISBN: 978-81-322-2798-4, 1-16.

Lee, S.W. (1984). Hankuk Sikpum Saltoesa (History of Korean Food and Society). *Kyomunsu. Seoul. Korea*, 168.

Li, H., Jiao, A., Xu, X., Wu, C., Wei, B., Hu, X. and Tian, Y. (2013). Simultaneous saccharification and fermentation of broken rice: an

enzymatic extrusion liquefaction pre-treatment for Chinese rice wine production. *Bioprocess and biosystems engineering* 36(8): 1141-1148.doi: 10.1007/s00449-012-0868-0.

Li, X.R., Ma, E.B., Yan, L.Z., Meng, H., Du, X.W., Zhang, S.W. and Quan, Z.X. (2011). Bacterial and fungal diversity in the traditional Chinese liquor fermentation process. *International journal of food microbiology* 146(1): 31-37.10.1016/j.ijfoodmicro.2011.01.030 .

Lim, E.A.Y., Panes, V.A. and Romero, G.O. (2006). Species identification and genetic diversity analysis by DNA fingerprinting of yeast isolates from Philippine rice wine starters. *Philippine Agricultural Scientist* 89(4): 326.

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.

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. doi:10.1016/j.ijfoodmicro.2017.06.012.

Lotong, N. (1985). *Koji Microbiology of fermented foods*, Elsevier Applied Science Publishers, London, 237-270.

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* 34:183–190. doi:10.1016/J.FOODCONT.2013.04.020.

Lv, X.C., Weng, X., Huang, R.L., Wen, Z., Rao, P.F. and Ni, L. (2012). Research on biodiversity of yeasts associated with Hongqu glutinous rice wine starters and the traditional brewing process. *Journal of Chinese Institute Food Science Technology* 12: 182-190.

Mao, A. A. (1998). Ethnobotanical Observation of Rice Beer '*Zhuchu*' Preparation by the Mao Naga Tribe from Manipur (India). *Bulletin Botanical survey of India* 40(1-4): 53-57.

Mao, Ashiho A. and N. Odyuo. (2007).Traditional fermented foods of the Naga tribes of North-Eastern, India. *Indian Journal of Traditional Knowledge* 6(1): 37–41.

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.

Mayo, B., T.C.C. Rachid, C., Alegría, Á., M.O Leite, A., S Peixoto, R. and Delgado, S. (2014). Impact of next generation sequencing techniques in food microbiology. *Current Genomics* 15(4): 293-309.

Moraes, P.M., Perin, L.M., Silva J.A. and Nero, L.A. (2013). Comparison of phenotypic and molecular tests to identify lactic acid bacteria. *Brazilian Journal of Microbiology* 44(1): 109-112. doi:10.1590/S1517-83822013000100015.

Moreira, N., Mendes, F., Hogg, T. and Vasconcelos, I. (2005). Alcohols, esters and heavy sulphur compounds produced by pure and mixed cultures of apiculture wine yeasts. *International Journal of Food Microbiology* 103(3): 285–294.

Murakami, H. (1972). Some problems in sake brewing. In *fermentation Technology, the proceedings of 4th International Fermentations Symposium, ed. G. Terui, pp. 639-643.* Kyoto, Japan.

Nam, Y.D., Lee, S.Y. and Lim, S.I. (2012). Microbial community analysis of Korean soybean pastes by next-generation sequencing. *International Journal of Food Microbiology*155: 36–42. doi:10.1016/j.ijfoodmicro.2012.01.013.

Nikkuni S., Karki T.B., Terao T., Suzuki C. (1996). Microflora of mana, a Nepalese rice *koji*. *Journal of Fermentation and Bioengineering* 81: 168–170. 10.1016/0922-338X (96) 87597-0.

Nisiotou, A.A. and Nychas, G.J.E. (2007). Yeast populations residing on healthy or Botrytis infected grapes from a vineyard in Attica, Greece. *Applied and Environmental Microbiology* 73: 2765e2768. doi:10.1128/AEM.01864-06.

Nout, M.J.R. and Aidoo, K.E. (2002). In Mycota: A comprehensive treatise on fungi as experimental systems and applied research, industrial applications ed. H. D. Osiewacz, Berlin: Springer-Verlag. 10: 23–47.

Nout, M.R. (2009). Rich nutrition from the poorest–Cereal fermentations in Africa and Asia. *Food Microbiology* 26(7): 685-692.doi:10.1016/j.fm.2009.07.002.

Oguntoyinbo, F.A., Tourlomousis, P., Gasson, M.J. and Narbad, A. (2011). Analysis of bacterial communities of traditional fermented West African cereal foods using culture independent methods. *International Jornal of Food Microbiology* 145: 205–10. doi:10.1016/j.ijfoodmicro.2010.12.025.

Otani, S. (1973). Alcoholic beverages in China II. Fermented Wine – Yellow – Wine. *Journal of Brewing Society of Japan* 68: 579-586.

Park, K.I., Mheen, T.I., Lee K.H., Chang, C.H., Lee, S.R. and Kwon, T.W. (1977). Korean *yakju* and *takju*. In: *The Proceeding and Symposium on Indigenous Fermented Foods*, GIAMI, Bangkok, November 21-27.

Pedersen, H. and Nielsen, J. (2000). The influence of nitrogen sources on the α-amylase productivity of *Aspergillus oryzae* in continuous cultures. *Applied Microbiology and Biotechnology* 53(3): 278-281.doi.org/10.1007/s002530050.

Pichyangkura, S. and Kulprecha, S. (1977) Survey of mycelial moulds in loogpang from various sources in Thailand. In: *The Proceeding and Symposium on Indigenous Fermented Foods*, November 21-27, GIAMI, Bangkok.

Pitt, J.I., Lantz, H., Pettersson, O.V. and Leong, S.l. (2013). *Xerochrysium* gen. nov. and *Bettsi*a, genera encompassing xerophilic species of *Chrysosporium*. *IMA Fungus* 4(2): 229-241.doi:10.5598/imafungus.2013.04.02.08.

Prakash, O. (1961) *Food and Drinks in Ancient India*. Munshi Ram Monoharlal Publishers, Delhi.

Prakitchaiwattana, C.J., Fleet, G.H. and Heard, G.M. (2004). Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. *FEMS Yeast Research* 4(8): 856-877.

Pretorius, I.S. (2000) Review: Tailoring wine yeast for the new millennium: novel approaches to the ancient art of wine making. *Yeast* 16: 675-729.

PrinsenGeerligs, H.C. (1896). Einige chinesische Sojabohnenpraeparate [some Chinese soybean preparations]. *Chemiker-Zeitung* 20(9): 67-69.

Pryce, T.M., Palladino, S., Kay, I.D. and Coombs, G.W. (2003). Rapid identification of fungi by sequencing the ITS1 and ITS2 regions using an automated capillary electrophoresis system. *Medical mycology* 41(5): 369-381.

Puerari, C., Magalhâes-Guedes, T.M. and Schwan, R.F. (2015). Physicochemical and microbiological characterization of chicha, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. *Food Microbiology* 46: 210–217.

Quigley, L., O'Sullivan, O., Beresford, T.P., Ross, R.P., Fitzgerald, G.F. and Cotter, P. D. (2012). High-throughput sequencing for detection of subpopulations of bacteria not previously associated with artisanal cheeses. *Applied and environmental microbiology* 78(16): 5717-5723.

Rampersad, S.N. (2014). ITS1, 5.8S and ITS2 secondary structure modelling for intra-specific differentiation among species of the *Colletotrichum gloeosporioidessensu lato* species complex. *Springerplus* 3: 684.

doi:10.1186/2193-1801-3-684.

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

Ray, R., Ghose, J. C. and Muwahhiddin, T. (1906). *The English Works of Raja Rammohun Roy*. Cosmo.

Roh, S.W., Abell, G.C., Kim, K.H., Nam, Y.D. and Bae, J.W. (2010). Comparing microarrays and next-generation sequencing technologies for microbial ecology research. *Trends in biotechnology* 28(6): 291-299. doi: 10.1016/j.tibtech.2010.03.001.

Romano, P., Capece, A. and Jespersen, L. (2006). "Taxonomic and ecological diversity of food and beverage yeasts," In: A. Querol and G.H. Fleet (Eds.) *Yeasts in Food and Beverages*, Berlin, Heidelberg: Springer Berlin Heidelberg, 13–53. doi:10.1007/978-3-540-28398-02.

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. doi:org/10.1080/09709274.2004.11905665.

Saelim, K. and Dissara, Y. (2008). Saccharification of cassava starch by *Saccharomycopsis fibuligera* YCY1 isolated from *Loog-Pang* (rice cake starter). *Songklanakarin Journal of Science & Technology*30: 65-71.

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.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution* 4(4): 406-425.

Sakai H., Caldo G.A. and Kozaki, M. (1983). Yeast-flora in red *burong-isda* a fermented fish food from the Philippines. *Journal of Agricultural Science* 28: 181–185.

Sakai, H. and Caldo, A.G. (1985). Microbiological and chemical changes in tapuy fermentation. *Journal of fermentation technology* 63(1): 11-16.

Samati, H. and Begum, S.S. (2007). *Kiad*-a popular local liquor of Pnar tribe of Jaintia hills district, Meghalaya. *Journal of Traditional Knowledge* 6(1): 133-135.

Sanchez, P.C. (1986). Traditional fermented foods of the Philippines. In: *Traditional Foods: Some Products and Technologies,* (Ed: Director, CFTRI). CFTRI, Mysore, 84-96.

Sanchez, P.C., L.S. Collado, C.L. Gerpacio and Lapitan, H.R. (1985). Village-level technology of processing coconut water vinegar. Phil. Agr., 68: 349-448.

Saono, S. and Basuki, T. (1978). The amylolytic, lypolytic, and proteolytic activities of yeasts and mycelial molds from ragi and some Indonesian traditional fermented foods. *Annales Bogorienses* 6: 207-219.

Saono, S., Gandjar, I., Basuki, T. and Karsono, H. (1974). Mycoflora of ragi and some other traditional fermented foods of Indonesia. *AnnalesBogorienses* (4): 187-204.

Saono, S., Hosono, A., Tomomatsu, A., Matsuyama, A., Kozaki, M and Baba, T. (1984). The preparation of *bremragi* – an improved method. *Proceedings of IPB – JICA* (July 31 – August 2) pp.152-158.

Savitri, and Bhalla, T.C. (2007). Traditional foods and beverages of Himachal Pradesh. *Indian Journal of Traditional Knowledge* 6: 17–24.

Scholz, M., Ward, D.V., Pasolli, E., Tolio, T., Zolfo, M. and Asnicar, F. (2016). Strain-level microbial epidemiology and population genomics from shotgun metagenomics. *Nat Methods* 13: 435–438.

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.

Shangpliang, H.N.J., Rai, R., Keisam, S., Jeyaram, K. and Tamang, J.P. (2018). Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. *Scientific Reports* 8: 1532 DOI.10.1038/s41598-018-19524-6

Shin, D.H., Kim, Y.M., Park, W.S. and Jae-Ho Kim, J.H. (2016). Ethnic fermented foods and beverages of Korea. In: *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (Ed: Tamang, J.P.), Springer, New Delhi. ISBN: 978-81-322-2798-4, 263-308.

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. 10.1081/FBT-120004198.

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.K., Singh, A. and Sureja, A.K. (2007). Traditional foods of monpa tribe of west kameng, Arunachal Pradesh. *Journal of Traditional Knowledge* 6(1): 25-36.

Soedarsono, J. (1972). Some notes on ‘ragitapé’ an inoculum for ‘tapé’ fermentation. *MajalahIlmuPertanian*1: 235-241.

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. (1996). *Handbook of Indigenous Fermented Food*, 2nd edition. Marcel Dekker, Inc., New York.

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.

Sugawara, E. (2010). Fermented soybean pastes *miso* and *shoyu* with reference to aroma. In *Fermented foods and beverages of the world* (pp. 233-253). CRC Press.

Sujaya, I.N., Amachi, S., Yokota, A., Asano, K. and Tomita, F. (2001). Identification and characterization of lactic acid bacteria in *ragi* tape. *World Journal of Microbiology and Biotechnology* 17: 349-357.doi: 10.1023/A:1016642315022

Sujaya, I.N., Nocianitri, K.A. and Asano, K. (2010). Diversity of bacterial flora of Indonesian *ragi tape* and their dynamics during the tape fermentation as determined by PCR-DGGE. *International Food Research Journal*17: 239-245.

Sukhumavasi, J., Kato, K. and Harada, T. (1975). Glucoamylase of a strain of *Endomycopsis fibuligera* isolated from mould bran (Look Pang) of Thailand. *Journal of Fermentation Technology* 53 (8): 559-565.

Surono, I.S. (2016). Ethnic fermented foods and beverages of Indonesia in: Tamang, J.P. (Ed.), Ethnic Fermented Foods and Alcoholic Beverages of Asia. Springer, New Delhi, 341-382.

Susan, S. E., Hohmann, S.L., Simmon, K. and Hanson, K.E. (2012). Internal transcribed spacer region sequence analysis using Smart Gene IDNS software for the identification of unusual clinical yeast isolates. *Medical Mycology* 50(5): 458–466

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. and Fleet, G.H. (2009). Yeasts diversity in fermented foods and beverages. In: *Yeast Biotechnology: Diversity and Applications*, Springer Netherlands, 169-198.

Tamang, J.P and Thapa, S. (2006). Fermentation dynamics during production of bhaatijaanr, a traditional fermented rice beverage of the Eastern Himalayas. *Food Biotechnology* 20(3): 251-261.

Tamang, J.P. (2010a). *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values*. CRC Press, Taylor & Francis Group, New York, ISBN: 9781420093247.295.

Tamang, J.P. (2010b). Diversity of fermented foods, In: Tamang JP, Kailasapathy K. (Eds.) Fermented Foods and Beverages of the World, CRC Press, Taylor & Francis Group, New York, 41–84.

Tamang, J.P. (2010c). Diversity of fermented beverages, In: Tamang J.P., Kailasapathy, K. (Eds.), Fermented Foods and Beverages of the World, CRC Press, Taylor & Francis Group, New York, 85–125.

Tamang, J.P. (2012). Plant-Based Fermented Foods and Beverages of Asia. In: Y.H . Hui and E. Özgül. Evranuz (Eds.) *Handbook of Plant-Based Fermented Food and Beverage Technology*, Second Edition, CRC Press, Taylor & Francis Group, New York, 49–90. ISBN: 978-1-4398-4904-0; eBook ISBN: 978-1-4398-7069-3. DOI: 10.1201/b12055-6.

Tamang, J.P. (2016). *Ethnic Fermented Foods and Alcoholic Beverages of Asia*. New Delhi: Springer India ISBN: 978-81-322-2798-4 doi:10.1007/978-81-322-2800-4.

Tamang, J.P. and Fleet, G.H. (2009). Yeasts diversity in fermented foods and beverages. In: *Yeasts Biotechnology*: *Diversity and Applications*, eds. T. Satyanarayana and G. Kunze. New York: Springer. 169–198.

Tamang, J.P. and Holzapfel, W.H. (1999). Microflora of fermented foods. *Encyclopedia of Food Microbiology 2*: 249-252.

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). CRC Press, Taylor & Francis Group, New York, 1-40**.** ISBN: 97814 20094954.

Tamang, J.P. and Sarkar, P.K. (1995). Microflora of marcha: an amylolytic fermentation starter. *Microbios* 81:115– 122

Tamang, J.P., and Fleet, G.H. (2009). Yeasts diversity in fermented foods and beverages, In T. Satyanarayana, and G. Kunze (Eds.) Yeasts Biotechnology: Diversity and Applications (New York, NY: Springer), 169–198. doi: 10.1007/978-1-4020-8292-49.

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., 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., Sarkar, P.K. and Hesseltine, C.W. (1988). Traditional fermented foods and beverages of Darjeeling and Sikkim - a review. *Journal Science Food Agricultural* 44(4): 375–385. doi:10.1002/jsfa.2740440410.

Tamang, J.P., Thapa, N., Savitri, and Bhalla, T.C. (2016b). Ethnic fermented foods and beverages of India. In: Tamang, J.P (Eds.), *Ethnic Fermented Foods and Alcoholic Beverages of Asia* pp. 17-72. Springer, New Delhi. ISBN: 978-81-322-2798-4.

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.

Tanaka, T. and Okazaki, N. (1982). Growth of mould on uncooked grain. *Hakkokogaku* 60: 11-17.

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.

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.

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.doi.org/10.1016/j.fbio.2015.05.002

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. *International Journal of Food Microbiology* 128: 268-273. doi:10.1016/j.ijfoodmicro.2008.08.020.

Thapa, S. and Tamang, J.P. (2004). Product characterization of kodokojaanr: fermented finger millet beverage of the Himalayas. *Food Microbiology* 21(5): 617-622.

Thapa, S. and Tamang, J.P. (2006). Microbiological and physico-chemical changes during fermentation of kodokojaanr, a traditional alcoholic

beverage of the Darjeeling hills and Sikkim. *Indian Journal of Microbiology* 46 (4): 333-341.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22(22): 4673-4680.

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.

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. doi:10.1016/j.ijfoodmicro.2004.08.011.

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.

Uchimura, T., Okada, S. and Kozaki, M. (1991). Identification of lactic acid bacteria isolated from Indonesian Chinese starter, "*ragi*". Microorganisms in Chinese Starters from Asia. *Journal of Brewing Society of Japan* 86(1): 55-61.

Urso, R., Rantsiou, K., Dolci, P., Rolle, L., Comi, G. and Cocolin, L. (2008). Yeast biodiversity and dynamics during sweet wine production as

determined by molecular methods. *FEMS yeast research* 8(7): 1053-1062.doi: 10.1111/j.1567-1364.2008.00364.x.

Vachanavinich, K., Kim, W.J. and Park, Y.I. (1994) Microbial study on *krachae*, Thai rice wine. In: Lee, C.H., Adler-Nissen, J. and Bärwald, G (Eds.),Lactic Acid Fermentation of Non-alcoholic Dairy Food and Beverages, Ham Lim Won, Seoul, 233-246.

Wang, H.L. and Hesseltine, C.W. (1970). Sufu and *lao-chao*. *Journal of Agricultural and Food Chemistry*, 18(4): 572-575.

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.doi:10.1128/AEM.00062-07.

Watson, D.C. (1993). Yeasts in distilled alcoholic beverage production. In *The Yeasts*, *vol. v yeast technology*, 2nd edition, eds. A.H. Rose and J.S. Harrison, 215-244. London: Academic Press.

Wei, D.L. and Jong, S.C. (1983). Chinese rice pudding fermentation: fungal flora of starter cultures and biochemical changes during fermentation. *Journal of fermentation technology* 61(6): 573-579.

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J.L. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18(1): 315-322.

Wickerham, L.J. (1951).*Taxonomy of yeasts*. *Technnical bulletin*, vol. 1029.United States Development of Agriculture, Wasington, D.C.

Wu, H., Watanabe, T., Araki, Y., Kitagaki, H., Akao, T., Takagi, H. and Shimoi, H. (2009). Disruption of ubiquitin-related genes in laboratory yeast strains enhances ethanol production during sake brewing. *Journal of bioscience and bioengineering* 107(6): 636-640.

Xie, G.F., Li, W.J., Lu, J., Cao, Y., Fang, H. and Zou, H.J. (2007). Isolation and identification of representative fungi from *Shaoxing* rice wine wheat *qu* using apolyphasic approach of culture-based and molecular-based methods. *Journal of Institute Brewing* 113: 272-279.doi: 10.1002/j.2050-0416.2007.tb00287.x.

Xu, W.B., Zhang, Q., Wen, F. A.N.G., Liao, W.B., Pan, B., Chang, H.S.U.A.N. and Chung, K.F. (2012). Nine new combinations and one new name of *Primulina* (Gesneriaceae) from South China. *Phytotaxa* 4(1): 1-8.

Xu, Y., Zhi, Y., Wu, Q., Du, R. and Xu, Y. (2017). *Zygosaccharomyces bailii* is a potential producer of various flavor compounds in Chinese *Maotai*-flavor liquor fermentation. *Frontiers in Microbiology* 8: 2609 doi: 10.3389/fmicb.2017.02609.

Yamamoto, S. (2016). Ethnic fermented foods and beverages of Cambodia. In: *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (pp. 237-262). Springer, New Delhi.

Yamamoto, S. and Matsumoto, T. (2011). Rice Fermentation Starters in Cambodia. *Japanese Journal of Southeast Asian Studies* 49(2): 192-213.

Yan, Y., Qian, Y., Ji, F., Chen, J. and Han, B. (2013). Microbial composition during Chinese soy sauce *koji*-making based on culture dependent and independent methods. *Food Microbiol*ogy 34: 189–195.

Yang, S., Choi, S.J., Kwak, J., Kim, K., Seo, M., Moon, T.W. and Lee, Y.W. (2013). *Aspergillus oryzae* strains isolated from traditional Korean *nuruk*: fermentation properties and influence on rice wine quality. *Food Science Biotechnol*ogy 22(2): 425-432. doi:10.1007/s10068-013-0097-6.

Yarrow, D. (1998). Methods for isolation, maintenance and identification of yeast. In: *The Yeast, a taxonomic Study*. (Ed: Kurtzman, C.P. and Fell, J.W.), Elsevier Science, Amsterdam, pp. 77-105.

Yokotsuka, T. (1985). Fermented Protein Foods in the Orient, with Emphasis on *Shoyu* and *Miso* in Japan, In: Wood. BJB (Eds.)Microbiology of Fermented Foods, *Elsevier Applied Science Publishers*, UK.

Yokotsuka, T. (1991). Non-proteinaceous fermented foods and beverages produced with *koji* molds. In: Arora, D.K, Mukerji, K.G. and Marth, E.H. (Eds.). *Handbook of Applied Mycology* vol. 3, Marcel Dekker, Inc., New York, 293-3283.

Zhang, Z., Chang, X. and Zhong, Q. (2008). Liquor Qu fungus system and enzymatic system character and microbial dynamic variety during vintage. *Liquor Making* 5: 24–29.

Zhao, K. and Chu, X. (2014). G-BLASTN: accelerating nucleotide alignment by graphics processors. *Bioinformatics* 30: 1384–1391. doi:10.1093/bioinformatics/btu047.

Zhu, Y. and Tramper, J. (2013). *Koji*–where East meets West in fermentation. *Biotechnology advances*31(8):1448-1457.doi:10.1016/j.biotechadv.2013.07.001.

Zott, K., Miot-Sertier, C., Claisse, O., Lonvaud-Funel, A. and Masneuf-Pomarede, I. (2008). Dynamics and diversity of non-*Saccharomyces* yeasts during the early stages in winemaking. *Internatioanl Jounal of Food Microbiology* 125: 197–203. doi:10.1016/j.ijfoodmicro.2008.04.001.