IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
Research Paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Production of Diverse Varieties of Amylolytic Starters In the North Bengal region For Sustainable Livelihood of Ethnic Tribes and Microbiology of Produced amylolytic Starters

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Abstract

Fermentation is an integral part of ethnic people of North Bengal for the preservation of raw substrate from spoilage as well as to preserve the viable forms of microbial consortia for the desired food development. Ethnic communities prepare different types of fermented foods, alcoholic beverages and amylolytic starters by using their traditional knowledge. These fermented foods they consume and sell for their sustainable livelihood. There are different types of fermented foods and alcoholic beverage in North Bengal region prepared by these ethnic population from the ancient times. There are various types of alcoholic starters in the North Bengal region, prepared by using glutinous rice as raw substrate and old starter culture which contains the microbial inocula for the fermentation and final product development. These amylolytic starters contains yeasts, molds and lactic acid bacteria their microbial consortia and dominance of yeasts and molds have been observed. All eight district of North Bengal contains different types of alcoholic starters with different microbial diversity. The molds plays an important role in the breakdown of polysccharides to disaccharides and yeasts plays an important role in the fermentation of monosachharides thereby producing alcohol and carbon di-oxide as final products and so the name alcoholic fermentation. *Marcha, dabai, chot, and ranu goti*, are traditionally prepared dried amylolytic starters used to produce various ethnic alcoholic beverages in this region. In the present study the phenotypic characterization gives the metabolic fingerprints of yeasts present in all alcoholic starters of North Bengal samples which showed major dominance of (yeasts). Scanning electron microscope (SEM) has been performed to reveal the surface structures of yeast isolates. The alcohol content of the fermented product ranges between from 6-6.5 %, and the alcohol tolerance is up to 17 % (v/v), pH ranges between 3.9 to 4.0. The present study reveals the microbial diversity among diverse variety of all eight types of alcoholic starters from eight districts of North Bengal as well traditional production process of alcoholic starters in all eight districts and how it promote sustainable livelihood of ethnic people in this region.

Keywords: Alcoholic Starters, Yeast, Molds, LAB, North Bengal, Microbial consortia, SEM, Alcohol Content, Sustainable Development, Ethnic Tribes, Rural Biotechnology

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 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Introduction

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 (Sha et al., 2018; Tamang et al. 2016). 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 2016; Sha et al., 2016; Sha et al., 2018). 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 (Sha et al 2018; Tamang et al., 2016). Fermented beverages and alcoholic drinks are socially and culturally acceptable products for consumption, drinking, entertainment, customary practices and religious purposes (Tamang 2010c).

Generally, when certain microbes contaminate different foods and drinks makes it is unfit for human consumption. However, there is a certain group of foods and drinks in which the presence of microbes makes them more preferable and beneficial for consumption and are known as fermented foods and beverages. Since the advent of civilization, various methods for the fermentation of both plant and animal products have been described, with the earliest records appearing in the Fertile Crescent (Middle East), dating back to 6000 BC. It is clear, however, that in earlier times the preparation of these fermented foods and beverages was performed in an artisan way and without any knowledge of the potential role of the microorganism involved. However, by the middle of the nineteenth century, two events changed the way in which food fermentation were performed and our overall understanding of the processes involved. First, the Industrial Revolution resulted in the concentration of large masses of populations in towns and cities. As a consequence, food had to be produced in large quantities, requiring the industrialization of the manufacturing process. Second, the emergence of microbiology as a science in the 1850s resulted in increased knowledge of the biological basis behind food fermentations, and the process being understood for the first time (Blandino et al., 2003).

Fermented foods and beverages are produced by the action of different microorganisms themselves (bacteria, yeast, and mycelia fungi) and their enzymes as well (Nout, 2003). Fermentation is defined as the enzyme-catalyzed, energy-yielding pathway in the cells which involves the breakdown of molecules such as glucose anaerobically. Now, when it comes to food preservation fermentation is one the oldest and the most economical method used in food preservation. Apart from preservation, fermentation adds up to some more benefits to certain foods and beverages which include, the prevention of food spoilage by microorganisms, enhancing the nutritional value of the foods and beverages through the synthesis of essential amino acids and vitamins during the process of fermentation, enhancing the digestibility of food that is often difficult to assimilate from a nutritional perspective (Simango, 1997) and fermentation also makes the products better in terms of taste, aroma texture, ethanol content and so on, detoxification and destruction of certain undesirable substances present in the raw foods such as phytates, tannins, and polyphenols (Gadaga et al., 1999) is also carried out during fermentation. Microorganisms carrying out fermentation may be the indigenous microflora on the substrate or be added externally as a starter culture. A starter culture is a microbial preparation containing a very high number of cells of one or more microbial species that are added to raw material to produce fermented food by accelerating and directing fermentation. North Bengal, is commonly referred to as the place located in the Northern part of West Bengal, India. The ethnic communities of the North Bengal region prepare and consume various indigenous fermented foods and beverages on a regular basis. *Marcha, dabai, chot, and ranu goti*, are

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traditionally prepared dried amylolytic starters used to produce various ethnic alcoholic beverages in this region. The present study aims to identify varieties of starters in North Bengal and to determine their microbial diversity.

In recent years, the most highlighted outcome of fermented food and drinks is their consumption as a source of probiotics. Probiotics are live beneficial microorganisms that when consumed in adequate amount provides health benefits to an individual. The most widely used probiotic bacteria are lactobacilli, a genus of lactic acid bacteria (LAB), and bifidobacteria (Assmann et al., 2006; Matthan et al., 2009). Since lactic acid bacteria are of both pathogenic and non-pathogenic nature, the non-pathogenic group generally contributes to the beneficial microbial population inhabiting the gastrointestinal (GI) tract in humans (Marteau et al., 2001; Haenel *et al*., 1975). Whereas, the only yeast genus that has been proven effective in double-blind studies is *Saccharomyces* (Czerucka et al., 2007). Yeasts are eukaryotic microorganisms and are found in natural habitats such as water, soil, air, and even on plant and fruit surfaces. Yeast ferments sugar produces secondary metabolites that inhibit the growth of mycotoxin-producing molds and has several enzymatic activities such as amylolytic, lipolytic, proteolytic, pectinolytic, glycosidic and urease activities (Aidoo et al. 2006).

Traditional practice of sub-culturing by back-sloping and preservation of essential native microbiota consisting of consortia of yeasts, molds, and bacteria, in the form of dry, fattened, or round balls, for alcoholic beverages production in South East Asia including the Himalayan regions of India, Nepal, Bhutan, and China is the worth wisdom of the ethnic people for centuries that must be preserved and sustained (Tamang et al., 2016; Sha et al., 2016; Sha et al., 2018).

Methodology

Survey

An extended survey was conducted in different parts of North Bengal seeking information about indigenous starters and other fermented foods and beverages. Mainly the tribal areas were visited including the local shops, markets, and haat bazaars. The survey includes all seven districts of North Bengal. The main aim of the survey was to collect information about different amylolytic starters, the raw materials used for their preparation, the process of preparation, and the way and occasion of consumption of different fermented foods and beverages prepared using these starters.

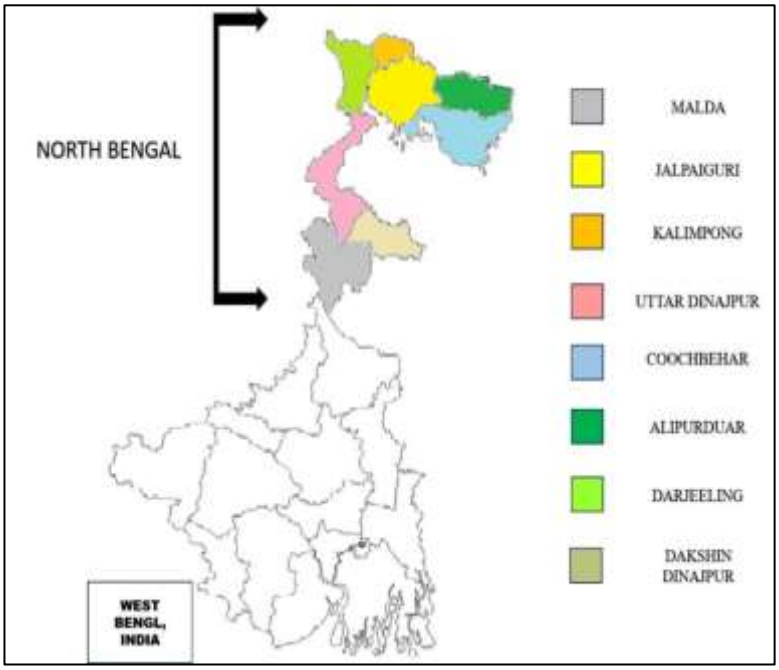

Figure 1: Map representing North Bengal.

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 Bengal, 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.

Sample Collection:

The samples were collected from all eight districts of North Bengal which includes Darjeeling, Coochbehar, Alipurduar, Kalimpong, Jalpaiguri, Uttar Dinajpur, Dakshin Dinajpur, and Malda. A total of 32 different samples of amylolytic starter were collected 4 from each district. These samples were then packed in gamma-irradiated sterile polybags and transported to the laboratory in a sterile condition for further analysis.

Isolation of Microorganisms

Yeast cells

One gram of the sample was homogenized with 9 ml of sterile distilled water and was kept for 1 minute. Thereafter, serial dilution was carried out in the same diluent. The same method was followed for all the samples collected respectively. Now, yeast isolation was done on yeast-malt agar (YM agar) supplemented with 10 IU ml^{-1}benzylpenicillin and 12 mg ml^{-1} streptomycin sulfate following the pour plate method. The plates were incubated at 28°C for 3 days in aerobic conditions and were observed for the appearance of yeast colonies. The purity of the isolates was checked by streaking again on YM agar plates followed by microscopic examination. Isolation of yeast strains was typically based on morphotypes and criteria including size, color, shape, and appearance of fully grown culture on growth media. Colonies were counted as colony forming units (cfu)/g sample. Identified strains of yeasts were preserved in 20% glycerol at −20◦C (Thapa et al., 2004).

Lactic Acid Bacteria

One gm of the sample was homogenized with 9 ml physiological saline for 1 min. Serial dilution of the sample in 0.85% NaCl solution was done, culturing them on de Man, Rogosa, and Sharpe (MRS) agar, a selective media supplemented with $CaCO_3$ for LAB followed by incubation at 30°C in Anaerobic gas Pack system (LE002) for 72 hours. Colonies are either selected randomly or all sampled if the plate contains less than 10 colonies, according to (Leisner et al., 1997). The colonies with a clear zone around them were considered LAB colonies. Pure culture of the isolates was obtained by streaking on MRS Agar without $CaCO_3$. The isolated LAB was preserved in MRS broth using 15% (v/v) glycerol at –80°C. Working culture will be preserved in MRS agar slants at –20°C.

Scanning Electron Microscopy of the Yeast and LAB isolates

Scanning electron microscopy has been used to study colony structures of the yeast and LAB isolates associated with amylolytic starters. Preparation of the yeast and LAB cultures for scanning electron microscopy (SEM) was done following the protocol for cultured microorganisms by Das Murtey and Ramasamy (2018) (Das Murtey et al., 2018). From the YPD agar, and MRS agar plates single colonies of yeast and LAB were taken and grown in YPD broth and MRS broth respectively

for 24 hrs at 25°C. One milliliter of the sample was centrifuged at 900 g for 2 min for pellet formation and resuspended in 5% glutaraldehyde solution prepared in 0.1 M phosphate buffer (pH 7.2) for fixation.30 minutes later the sample was centrifuged, the supernatant was discarded and the pellet was washed twice in 0.1M phosphate buffer. The pellet was resuspended in 1% osmium tetroxide prepared in 0.1M phosphate buffer. Sample dehydration was then carried out using ethanol series of 35, 50, 75, and 95%, absolute ethanol, and hexamethyldisilazane (HDMS) for 30 minutes per step, centrifuging and discarding the supernatant in each case. Lastly, the second HDMS was discarded and the sample was left to dry overnight in a desiccator.
Now, the dehydrated yeast sample was mounted on plain aluminum stubs using carbon double surface adhesive and coated with a 5 nm gold-palladium (80:20) layer using a Gold Sputter Coater (BIO-RAD Polaron Division, SEM coating system, United Kingdom) and observed under a constant accelerating voltage of 5 kV under a JEOL scanning electron microscope type 5510 (JEOL, Tokyo, Japan).

Results

Survey

The survey conducted was very informative and a good experience. From the survey, it was found that people living in tribal areas prepared these starters on a regular basis. Some of the tribal communities that prepared these starters are Adivasi, Rava, Toto, Santhal, Saibo, and Uraon of different regions of North Bengal. These starters were used by them for the preparation of various alcoholic beverages such as Tongba, haria, and jaanr. The beverages prepared were consumed by them and also sold in the local haat bazaars and shops for their living. Now, not only the beverages but even the starters were sold by these people at an affordable price. People from different places visited these local markets and bought the starters for their uses. So, preparation of the starter and selling proved to be a source of income for these people living in tribal areas.
Traditional practice of sub-culturing by back-sloping and preservation of important indigenous microbial consisting of consortia of yeasts, molds, and bacteria (LAB), in the form of dry, fattened, or round balls, for alcoholic beverages production in North Benagl, including the Himalayan regions of India, Nepal, Bhutan, and China is the worth important wisdom of the ethnic people for centuries. Ethnic fermented foods have been prepared for many centuries and preparation process varies across the country to country. In North bengal India fermented foods and alcoholic beverages are been fundamental part of ethnic heritage. One of the traditional oldest and most cost-effective methods for production and preserving food is fermentation (Tamang et al., 2016; Sha et al., 2018).

The traditional method of preparation of these starters is discussed in detail in the flowcharts below:

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Research Paper , UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

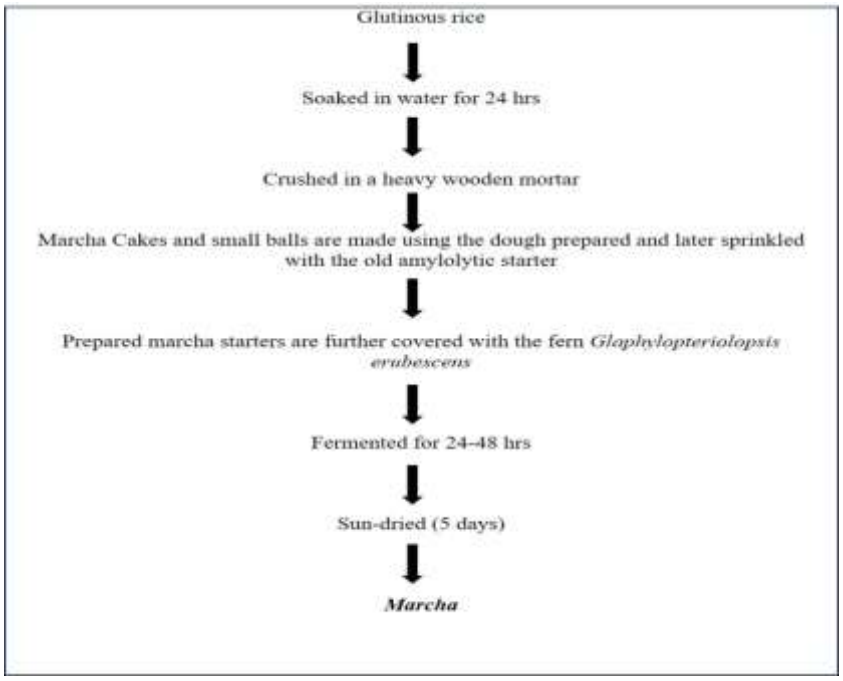

Figure 2: Marcha of Darjeeling and Kalimpong.

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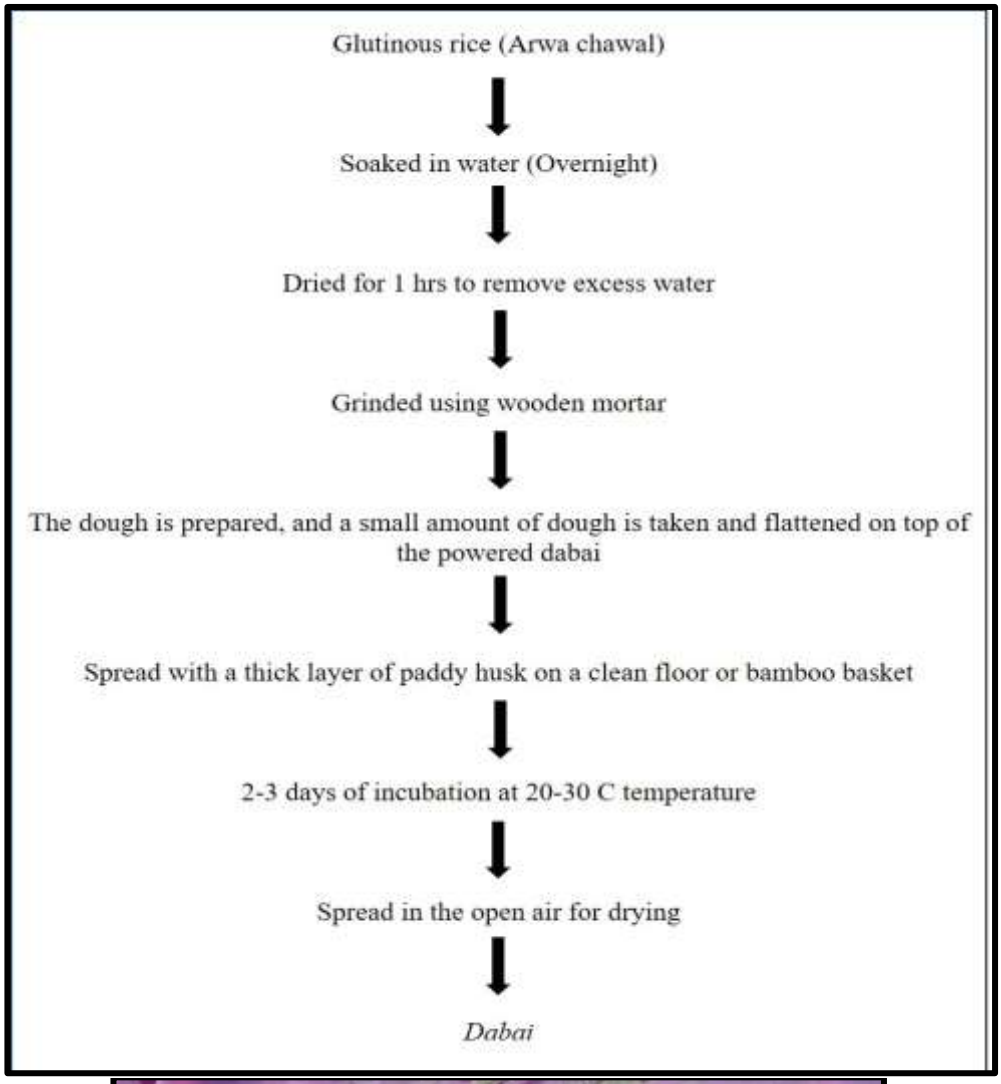

Figure 3: Dabai of Alipurduar and Jalpaiguri.

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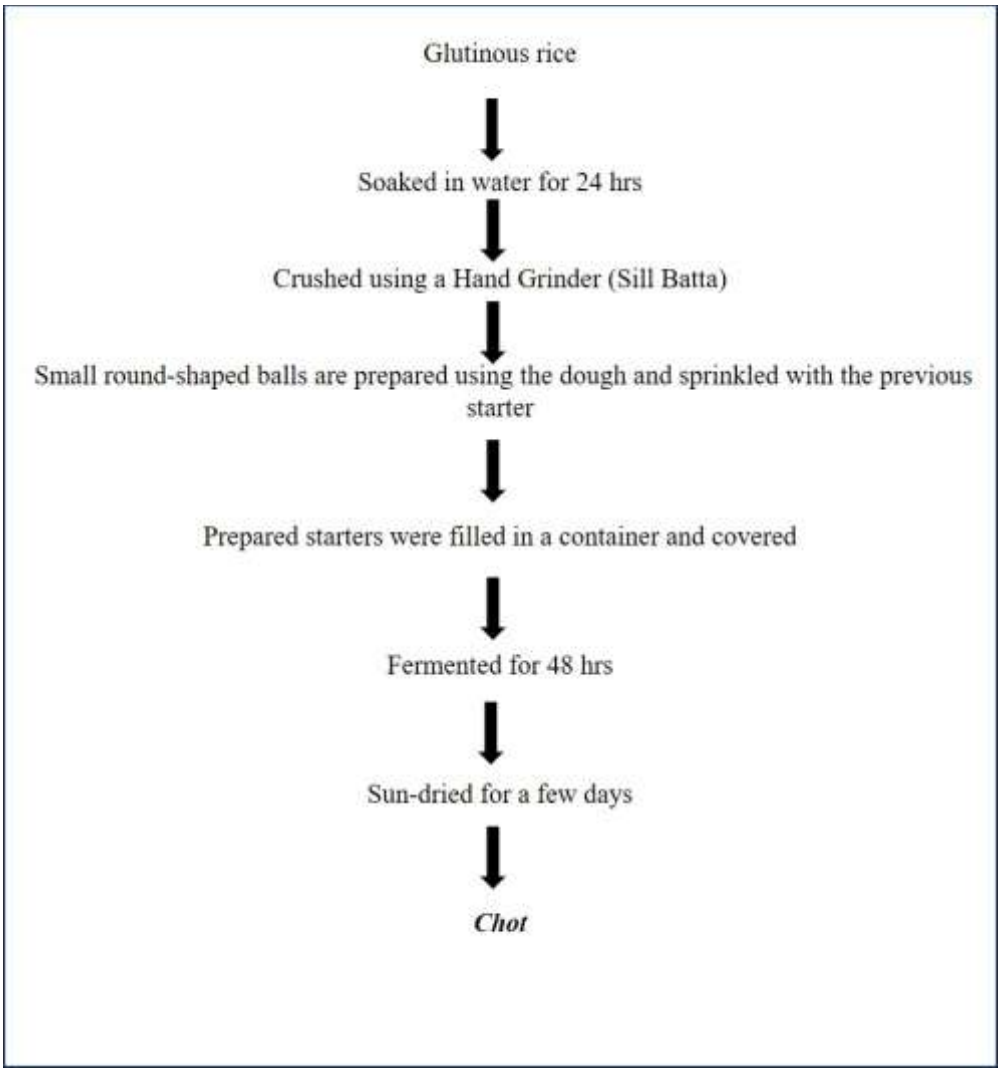

Figure 4: Traditional method of preparation of ethnic starter culture *Chot* of Coochbehar

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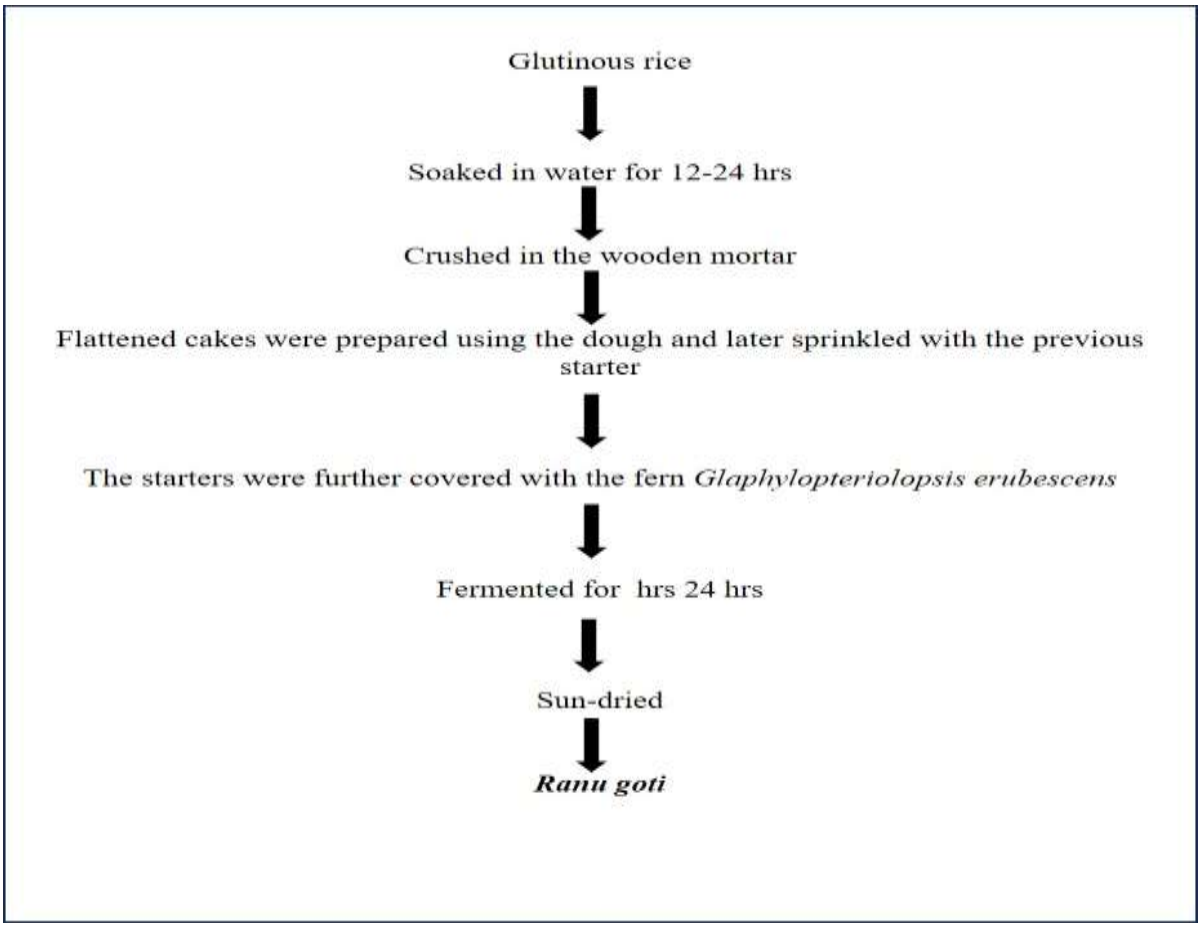

Figure 5: Ranu goti of Uttar Dinajpur, Dakshin Dinajpur

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Microbial Analysis:

A total of 28 starter samples were collected from all 8 districts of North Bengal, 4 from each district. Further, these samples were used for the isolation of yeasts and Lactic Acid Bacteria.

Table 1: Representation of the Samples.

Sample (Starter)	Number of Samples (n)	Districts of North Bengal	Common/local name of the sample	pH	Dominant Microorganism	Presence of Lactic Acid Bacteria (LAB)
cfu/g x 10^6	4	Darjeeling	*Marcha*	3.5	Yeast	Yes
	4	Coochbehar	*Chot, Dabai*	3.7	Yeast	Yes
	4	Alipurduar	*Dabai*	3.6	Yeast	No
	4	Kalimpong	*Marcha*	3.6	Yeast, Fungi	No
	4	Jalpaiguri	*Dabai, Ranu goti*	3.8	Yeast	Yes
	4	Uttar Dinajpur	*Ranu got, dabai,*	3.5	Yeast, Fungi	No
	4	Dakshin inajpur	*Ranu goti*	4.1	Yeast	No
	4	Malda	*Ranu goti*	4	Yeast, Fungi	No

Isolation of Microorganisms

A total of 140 yeasts were isolated from the 28 different starter samples of North Bengal. Here, 20 yeasts were isolated from the Darjeeling sample, 30 from the Coochbehar sample, 30 from the Alipurduar sample, 10 from the Kalimpong sample, 15 from the Jalpaiguri sample, 10 from the Uttar Dinajpur sample, 15 from Dakshin Dinajpur and 10 from Malda sample.

Figure: Amylolytic Starters of North Bengal

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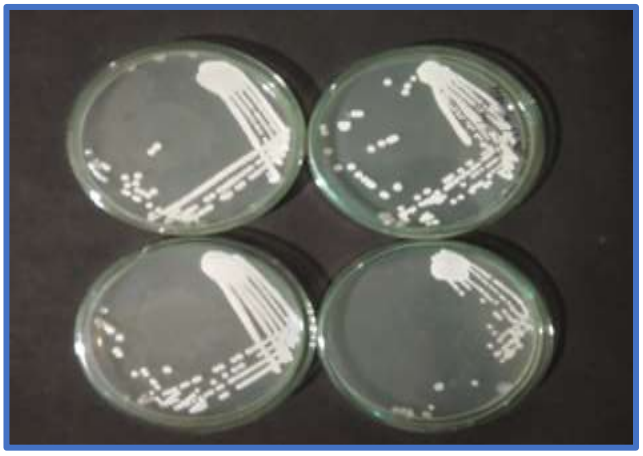

Figure 6: Isolated Yeast colonies.

Strain code	Sugars Fermented								Identified strains
	Glucose	Rhamnose	Galactose	Sucrose	Maltose	Lactose	Trehalose	Arabinose	*Saccharomyces cerevisiae*
AD-1	+	+	+	+	+	-	-	+	*Wickerhamomyces anomalus*
AD-2	+	+	+	+	-	-	+	-	*candida glabrata*
AD-3	+	-	+	+	+	-	+	-	*Kluyveromyces marxianus*
AD-4	+	+	+	+	+	-	-	+	*Saccharomycopsis fibuligera*
CY-1	+	+	+	+	+	+	+	+	*Pichia guilliermondii*
CY-2	+	+	+	+	+	+	+	+	*Candida glabrata*
CY-3	+	+	+	+	+	+	+	+	*Pichia tropicalis*
JY-1	+	+	+	+	+	-	+	+	*Pichia anomala*
JY-2	+	+	+	+	+	-	+	+	*Isatchenkia*
KY-1	+	+	+	+	+	+	+	+	*Wickerhamomyces anomalus*
KY-2	+	+	+	+	+	-	+	-	*Isatchenkia*
KY-3	+	+	+	+	+	-	-	-	*Pichia tericola*
DY-1	+	+	+	+	+	+	+	-	*Saccharomyces cerevisiae*
DY-2	+	+	+	+	+	+	+	+	*Isatchenkia*
DY-3	+	+	+	+	+	+	+	+	*Pichia anamala*

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DY-4	+	+	+	+	+	+	+	+	*Candida glabrata*
UDY-1	+	+	+	+	+	+	-	-	*Isatchenkia*
UDY-2	+	-	+	-	+	+	+	+	*Saccharomyces cerevisiae*
UDY-3	+	+	+	+	-	-	-	+	*Pichia anamala*
DDY-1	+	+	+	+	+	+	-	+	*Saccharomycopsis fibuligera*
DDY-2	-	+	-	-	+	+	+	-	*Saccharomycopsis fibulige*

Sugar Fermentation of yeast isolates of alcoholic starters of North Bengal:
positive results and - denotes negative resultes)
(+) sign = sugar fermented by yeast isolate
(--)sign=sugar not fermented by yeast isolate

Results of TPC shows the dominance of yeasts in terms of cfu/gm follwed by molds, LAB, TVC and Bacilli.

Microscopic studies of yeast isolated from amylolytic starters of North Bengal:

Product	**Log cfu /gm**					
Dabai	**Place of collection**	**Yeast**	**Moulds**	**LAB**	**TVC**	**Bacilli**
	Matigara Haat (n=3)	8.60 ±0.3	8.1 ±0.3	8.1 ±0.3	8.60 ±0.3	7.1 ±0.03
	Bagdogra village (n=3)	8.6 ±0.3	8.1 ±0.6	8.1 ±0.6	8.47 ±0.0	7.1 ±0.06
	Totopara (n=3)	8.7 ±0.3	8.0 ±0.3	8.1 ±0.0	8.60 ±0.0	7.0 ±0.4
	Malda (n=3)	8.6 ±0.0	8.17 ±0.3	8.2 ±0.0	8.70 ±0.0	7.1 ±0.6
	Sonapur (n=3)	8.7 ±0.3	8.0 ±0.0	8.20 ±0.0	8.60 ±0.3	7.2 ±0.5

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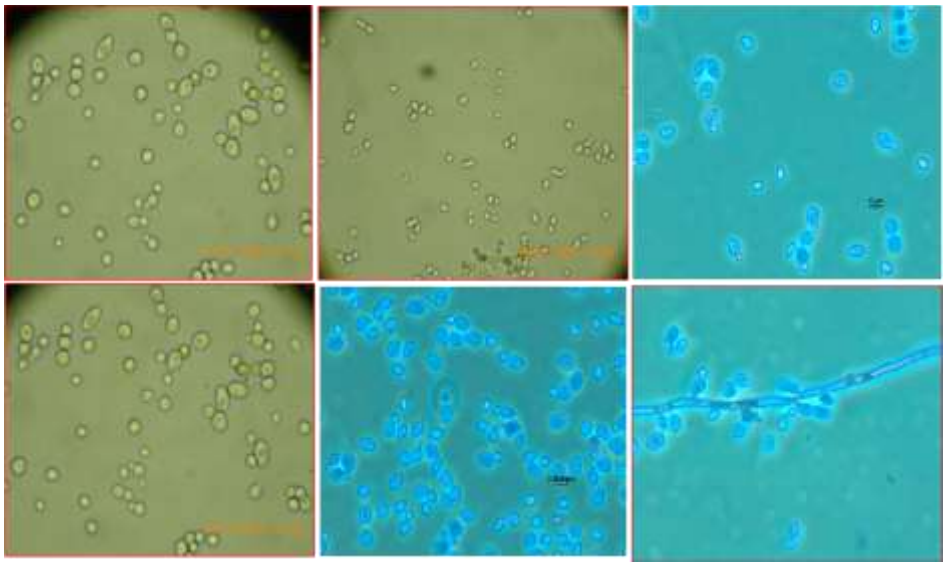

Figure: 7 Yeast morphology under 100X Binocular Microscopy

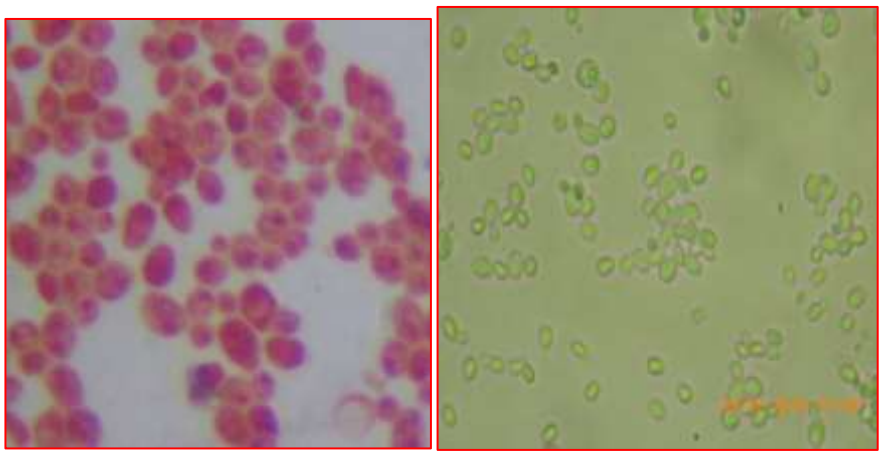

Figure: 8 Microscopic view of ascospores of *Saccharomyces cerevisiae* and *Candida glabrata* under 100X gigital binocular Microscope.

Lactic Acid Bacteria

Although these starters were found to be yeast-dominant, few Lactic Acid Bacteria (LAB) were isolated from starters of Darjeeling, Jalpaiguri, and Coochbehar. A total of 10 LABs were isolated i.e., 4 from the Darjeeling sample, 2 from the Jalpaiguri sample, and 4 from the Coochbehar sample. However, there may be LAB present in the samples of Alipurduar, Kalimpong, Uttar Dinajpur, Dakshin Dinajpur, and Malda, but due to high Yeast dominance in samples of these places, it was not possible to indicate and isolate them or LAB was absent in these samples.

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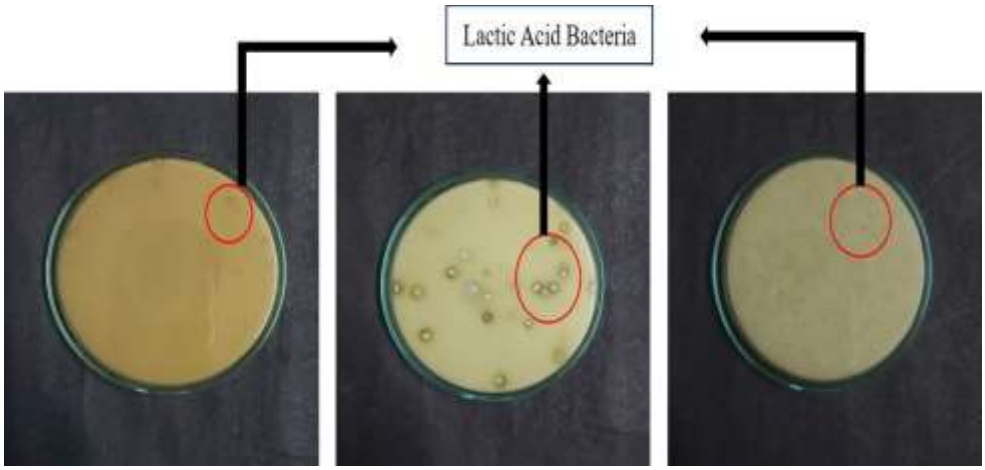

Figure 9: Lactic Acid Bacteria.

There are different types of lactic acid bacteria present in the alcoholic starters. They play an important role in the fermentation process as well as flavour development by synthesizing the lactate. These LAB play very important role in bio-preservation of amylolytic starters in a natural way by producing bacteriocins, nisin and lactate. Which inhibit the growth of closely related pathogenic enteric bacteria in the alcoholic starters, ultimately preserves the starters for longer time period and also very mcuh important for the flavour development in the fermented final product.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was performed to analyze yeast and LAB isolates and investigate differences in cell morphology. The SEM images can be seen in Figure 8. The shape of the Yeast cells varied from spheroid to oval and LAB were found to be of rod shape.

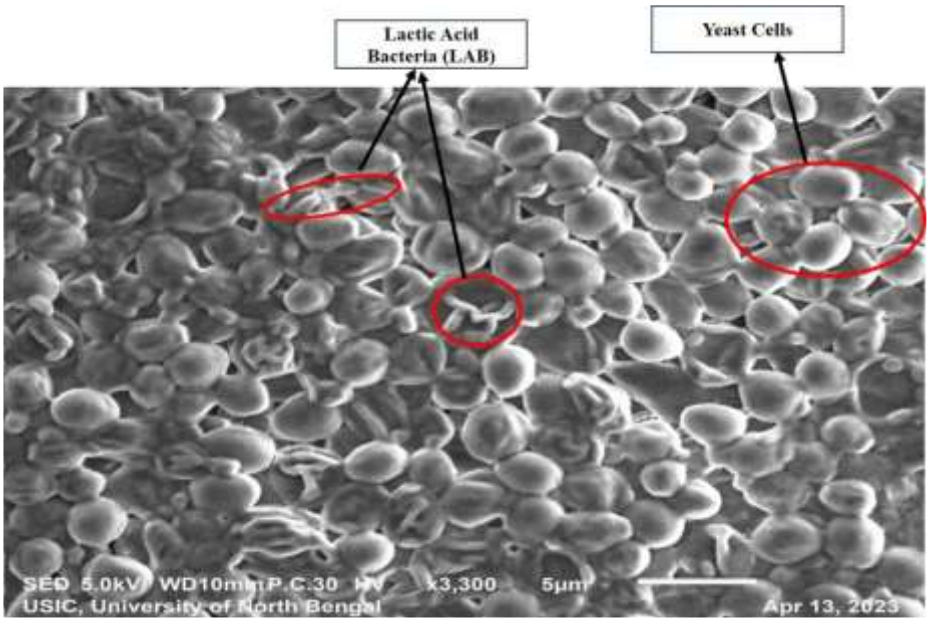

Figure 10: Scanning Electron Microscopy of mixed culture of Yeast and Lactic Acid Bacteria (LAB).

Discussion

Indigenous Asian ethnic communities have innovated the traditional knowledge of 'ethno-microbiology' "rural biotechnology" for production of organoleptically preferred and healthy

fermented food products. One of such excellent practices made by ethnic people with 'rural biotechnology' was preparation alcoholic amylolytic starter culture in North Bengal and some regions in the Indian subcontinent (Sha et al., 2018 and Sha et al., 2019). The production and sell of alcoholic starters is related with the culture and sustainable livelihood of ethnic people (Rava, Toto, Adivasi, Rajbansi, Limboo, Rai, Tamang, Lepcha, Bhutia, Limboo) of North Bengal (Sha et al., 2012; Sha et al., 2019).

Ethnic fermented foods by native or selective (starter based) microbial consortia contribute a important role in fermentation and product development they naturally convert the polysaccharides in to monosaccharides and ferment them in to desired product they may play vital role in dietary intervention as it biochemically changes the primary food matrix, enhances food with vitamins, minerals proteins, essential amino acids, essential fatty acids,vitamins, enhances the sensory properties and enhances the shelf-life of fermented foods (bio-preservation), and increases the digestibility of whole grains (Blandino et al., 2003; Obadina et al., 2013; Tamang et al., 2016). Yeasts, Lactic acid bacteria (LAB), acetic acid bacteria, Molds are the most dominant microorganisms responsible for fermentation; however, *Bacillus*, an some filamentous fungi, and other bacteria may also grow there and paly an vital role in final product development. Secondary metabolites are bio active compounds synthesized during fermentation of cereal-based foods also play an important role and exerts heath attributes (Long-Smith et al., 2020; Sandhu et al., 2017; Sha et al.,2016; Sha et al., 2018). Several studies showed that the use of lactic acid bacteria (LAB) as a functional starter, and cereal substrates (prebiotcs) use as media or substrate for the growth of probiotic microorganisms (Coda et al., 2011, Dongmo et al., 2017, Jung et al., 2019, Ogodo et al., 2019). However, there are many studies to show about the development of multi-grain based foods fermented by completely functional yeasts or probiotic yeast.

Recent studies have shown that the major predominnat genera *Issathenkia, Candida, Torulaspora, Kluyveromyces, Pichia, Candida, Whikheromyces* which have the ability to enhance immune system, produce B-vitamin complex, lower cholesterol, and enzymes as well as antimicrobial activity, against intestinal enteric pathogenic microorganisms. Moreover, probiotic yeast plays an important role in alcoholic fermentation and provides various health attributes (Tamng et al., 2016 and Sha et al., 2018).

There are different types of lactic acid bacteria present in the alcoholic starters. They play an important role in the fermentation process as well as flavour development by synthesizing the lactate. These LAB play very important role in bio-preservation of amylolytic starters in a natural way by producing bacteriocins, nisin and lactate. Which inhibit the growth of closely related pathogenic enteric bacteria in the alcoholic starters, ultimately preserves the starters for longer time period and also very much important for the flavour development in the fermented final product (Ghatani et al., 2017).

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. 2016). 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 producersyeastsCandida glabrata, Debaryomyces hansenii, Wickerhamomyces ciferrii, Saccharomycescerevisiae, Meyerozyma guilliermondii and Dekkera bruxellensis* (Sha et al., 2016;

Sha et al., 2017). *Aspergillus oryzae* isolated from Korean nuruk improves fermentation properties and rice wine quality (Yang et al. 2013). In the production of fermented food bacteria play dominant and important roles. LAB is the most abundant bacteria presents in the fermented foods among all the bacteria but some *Bacilli*, *Micrococcae* are also present in the fermented foods. The LAB contains of *BhaatiJaanr* and *Kodo ko Jaanr*are 10^4 to 10^6cfu/g, 5.9 cfu/g. The LAB load of *Haria* is 8.20±0.0 cfu/ml and the *Dabai* is about 8.20±0.0cfu/ml, which indicates that the LAB load of *Haria and Dabai* is greater than the LAB load of similar mild alcoholic beverages of Sikkim and Darjeeling Region. The LAB load of *BhaatiJaanr* and *Kodo Ko Jaanr* is less than the yeast load. Similarly, the LAB load of *Haria* is less than its yeast load. Lactic Acid Bacteria provides probiotics and prebiotic effect, increases lactose digestion, and prevent food borne pathogenic contamination (Tamang et al., 2016). The inability to utilize starch by *Pediococcuspentaceus* and *L.bifermentans* indicates that they do not play important role in the hydrolysis of the starch from the substrates during Kodo Ko Jaanr fermentation, however, probably they helps in flavour and taste developments of the product Similarly Dabai contains LAB microflora which might be playing similar role in the product development like other mild alcoholic beverages. In the present study LAB were isolated however they were not identified and preserved for further studies.

Conclusion

Traditional fermented foods and alcoholic beverages are attractive alternatives to fulfil the nutritional and food requirements of large number of people. In this chapter we have compiled all the information regarding traditional method of fermented food and alcoholic beverages of North East India and their method of preparation, consumption, sales, ethnic significance, microbial diversity, and functionality. These fermented foods and alcoholic beverages are related with the livelihood of the ethnic tribes of the entire region of North East India and North Bengal. Due to the indigenous knowledge of ethnic tribes and the new methods introduced by the immigrants arriving from other states of India, a wide variety of fermented foods are produced in North Bengal. These fermented foods and alcoholic beverages have the potential to grow into a small to medium sized industry, if proper scientific and technical support is extended to the existing indigenous practices of home-based fermentation. Ancient people, throughout the world are real bio-technologists, having independently discovered the value of fermenting food as a sustainable and cost-effective means of preservation, improving nutritional quality as well as enhancing sensory properties. The fermentation of dairy products, pulses, cereals, and other substrates to produce fermented foods and mild alcoholic beverages with health-promoting properties is indigenous to many regions of world. The ethnic communities of North East India and North Bengal use to ferment various raw materials naturally by using their home-based traditional knowledge and produce, hence ethnic people are using a mechanism of rural biotechnology for the preservation of their agricultural harvest as well as for the production of various fermented foods and alcoholic beverages for their consumption and livelihood. In this paper we have studied the production process of alcoholic starters of North Bengal with the traditional knowledge of Rural Biotechnology used by ethnic tribes for their sustainable development and livelihood and microbiology of the alcoholic starters. The isolated yeasts strains will be used for development of new starter culture for beer industry as well development of yeasts probiotic and probiotic drinks.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Figure:11 Amylolytic starters produced by ethnic tribes of North Bengal for traditional brewing

Funding
This study is funded by Science and Engineering Research Board (SERB) Department of Science and Technology, Government of India for granting research project "SRG/2021/002035" dated 30 December 2021.

Acknowledgments
SPS gratefully acknowledged the financial support of Science and Engineering Research Board (SERB) Department of Science and Technology, Government of India for granting research project "SRG/2021/002035" dated 30 December 2021.

Contributions
SPS, AS, SPSa and KG was involved in developing the idea of review, drafting manuscript, and finalizing manuscript. All authors contributed to the article and approved the submitted version.

Ethical Approvals
This study does not involve experiments on animals or human subjects.

Data Availability
All the raw data of biochemical and phenotypic tests are available with the authors and shall be provided upon request.

Conflicts of Interest
The authors report no financial or any other conflicts of interest in this work.

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