Microbiological Studies of *Haria*, a Traditional RiceFermented Alcoholic Beverage of West Bengal

Thesis Submitted for Master of Philosophy (M.Phil) Degree in Microbiology

of

Sikkim University

Shankar Prasad Sha

M.Phil. 4th Semester

Roll No: 10PDMB04; Regn. No: 10SU2086

Session: 2010-2011

Department of Microbiology, School of Life Sciences

Sikkim University

Sikkim, Tadong -737102, India

सिक्किम विश्वविद्यालय
(भारतके संसदके अधिनियमद्वारा स्थापित केंद्रीय विश्वविद्यालय)
SIKKIM UNIVERSITY
[A Central University established by an Act of Parliament of India in 2007]

DECLARATION

I declare that the thesis entitled "**Microbiological Studies of *Haria*, a Traditional Rice Fermented Alcoholic Beverage of West Bengal**" submitted for the award of **Master of Philosophy (M.Phil.)** Degree in **Microbiology** of **Sikkim University** is my original work. The content of this thesis is based on the experiments which I have performed myself. This thesis has not been submitted for any other degree to any other University.

The content of this M.Phil thesis has been subjected to the Anti-Plagiarism Software (Ephorus) and it was found satisfactory.

Date : 30th June 2012.

Shankar Prasad Sha.

Shankar Prasad Sha
Roll No: 10PDMB04
Regn .No: 10SU2086

We recommend that the thesis be placed before the examiners for evaluation.

29/6/12

Dr H K Tiwari
(Coordinator)
ASSISTANT PROFESSOR
Department of Microbiology
School of Life Sciences
SIKKIM UNIVERSITY
6th Mile Samdur P.O. Tadong-Sikkim

Dr Nagendra Thakur
(Assistant professor)
ASSISTANT PROFESSOR
Department of Microbiology
School of Life Sciences
SIKKIM UNIVERSITY
6th Mile Samdur P.O. Tadong-Sikkim

६ माइल, सामदुर, पीओ तादुङ–७३७१०२, गान्तोक, सिक्किम, भारत
फोनः00–९१–३५९२–२५१०६७, २५१४३८, फ्याक्स–२५१४३८
6th Mile, Samdur, PO Tadong 737102, Gangtok, Sikkim, India
Phones : 00-91-3592-251067, 251438, Fax - 251438
website : www.sikkimuniversity.in

सिक्किम विश्वविद्यालय
(भारतके संसदके अधिनियमद्वारा स्थापित केंन्द्रीय विश्वविद्यालय)
SIKKIM UNIVERSITY
[A Central University established by an Act of Parliament of India in 2007]

Certificate

This is to certify that the thesis entitled "**Microbiological Studies of *Haria*, a Traditional Rice Fermented Alcoholic Beverage of West Bengal**" submitted to the **Sikkim University** for the award of **Master of Philosophy (M.Phil.) Degree in Microbiology** embodies the results of bonafide research work carried out by **Mr Shankar Prasad Sha** under my guidance and supervision. No part of the thesis has been submitted for any other degree, diploma, associateship and fellowship.

All the assistance and help received during the course of the investigation have been acknowledged by him.

Dr. Nagendra Thakur

(Supervisor)

Department of Microbiology

Place: Gangtok, Sikkim
Date: 30 th June 2012.

ASSISTANT PROFESSOR
Department of Microbiology
School of Life Sciences
SIKKIM UNIVERSITY
6th Mile Samdur P.O. Tadong-Sikkim

६ माइल, सामदुर, पीओ तादुङ–७३७१०२, गान्तोक, सिक्किम, भारत
फोनः००–९१–३५९२–२५१०६७, २५१४३८, फ्याक्स–२५१४३८
6th Mile, Samdur, PO Tadong 737102, Gangtok, Sikkim, India
Phones : 00-91-3592-251067, 251438, Fax - 251438
website : www.sikkimuniversity.in

ACKNOWLEDGEMENT

With deep sense of gratitude, I would like to thank my esteemed supervisor Dr. Nagendra Thakur, Assistant Professor, Department of Microbiology, Sikkim University to whom I owe my entire effort. Without his keen supervision, able guidance, intellectual acumen, academic co-operation and invaluable suggestions, this work would not have been possible. I am deeply indebted to him for her gesture.

I take this opportunity to acknowledge my sincere gratitude to Honourable founding Vice-Chancellor of Sikkim University Prof. Mahendra P. Lama, for his encouragement, support and providing us with the requisite infrastructure.

I would like to acknowledge my deep senceo of gratitude to Prof.(Dr). Jyoti Prakash Tamang, Registrar of Sikkim University and professor, Department of Microbiology, for his encouragement, constant support, guidance and blessings.

I would also like to extend my warm and sincere thanks to Dr. Hare Krishna Tiwari, Associate professor, Dr. Bimala Singh, Assistant Proffessor, Dr. Buddhiman Tamang, Assistant Professor, Department of Microbiology, Sikkim University for their endless help and advice. In this context I would like to offer my sincere thanks to Mr. Gagan Sen Chettri, Laboratory Assistant, for his help and support.

The university authority and Department of Microbiology have given me ample support while nourishing the ideas of creating thesis. It has provided us with Hi tech laboratory and all the necessary technical items and books. I would like to thank Ms. Kriti Ghatani my best friend and always helped and encourage me during my M.Phil dissertation. I would also thankful to Mrs. Pramila Koirala for her help.

I am highly thankful to Miss Nikki Kharel, Research scholar, Food Microbiology Laboratory for providing us some microbial cultures for our study.

Credit also goes to my friends and all the students of Department of Microbiology for their direct and indirect help during the course of my dissertation work and during the preparation

phase of my thesis. I also express my gratitude to Mr. K .K Yadav for his constant support, blessings and valuable teaching during my post graduation at the University of North Bengal.

I am indebted to my Parents, late Smt. Shanti Devi and Sri Ram Prasad Sha, my family who stood behind me in favour of my decision, without thinking twice of the repercussion. They have always been a continuous source of encouragement and motivation.

I would also like to thank the Faculty members and Non teaching staff of Department of Chemistry and Department of Horticulture and Floriculture for technical support.

Again a special credit goes to villagers for necessary information and indigenous knowledge they provided during sample collection and documentation of traditional knowledge of *Haria* and *Dabai* preparation.

Last but not the least; I would like to thank all those who help me directly or indirectly in completing the thesis.

Date: 30/6/12

Shankar Prasad Sha.

Shankar Prasad Sha

CONTENTS

Chapter	Page Number
1 .INTRODUCTION	1-5
2. REVIEW OF LITERATURE	6-18
3. MATERIALS AND METHODS	19
3.1. Culture Media Used	20-21
3.2. Reagents	20-21
3.3. Method	22
3.3.1Survey and documentation oftraditional knowledge of *Haria*preparation.	22- 23
3.3.2.Mode of consumption.	23-24
3.3.3. Socio-economic significance	24
3.3.4.Socio-cultural significance	25
3.3.5. Collection of samples	25
3.3.6 Microbiological analysis	26
3.3.7. Isolation of Microorganisms	26
3.3.8 .Enumeration of Bacilli	26-27
3.3.9. Enumeration of TVC	27
3.3.10.Biolog identification system	27
3.4. Characterizationof yeast	28
3.4.1.Cell morphology	28
3.4.2. Pseudo and true myecelium	28
3.4.3 Characteristics of ascus and ascospores	28
3.4.4. Reduction of nitrate	29
3.4.5 Growth of yeast at 37° C	29
3.4.6.. Sugar fermentation	29

3.4.7. Sugar assimilation 29

3.4.8. Determination of important properties of yeast isolates of *Haria* and *Dabai*

(a) Ethanol tolerance 30

(b) Amylolytic activity 30

(c) Characterization of pathogenic contamination of *Haria* 30-31

3.5.Characteristics of bacterial isolates 32

(a) Cell Morphology 32

(b) Catalase Test 32

3.6.Biochemical analysis 32

(a) pH 32

(b)Titrable Acidity 32

(c) Moisture Content 33

(d) Alcohol Content 33-34

4. RESULTS 34

4.1.Traditional method of preparation of *Haria* 35

4.1.1. Method of preparation of *Haria* 35

4.1.2. Flow sheet of *Haria* preparation 36

4.1.3. Traditional method of preparation of *Dabai* 37

4.1.4 Flow sheet of preparation of *Dabai* 38

4.1.5. Similar products 39-42

4.2. Ethnic importance 42-43

4.3. Myths and Belief about *Haria* 43

4.4. Experimental 44

4.4.1. Microbiological Analysis of *Haria* 44

4.4.2. Microbial load of *Haria* 44

4.4.*3 Microbial* load of *Dabai* 47

4.4.4. Biolog identified yeast strains 49

4.4.5.isolation of microorganisms 49

4.4.6.Colony morphology of moulds 50

4.4.7. Nitrate reduction. 50

4.4.8. Growth at 37^0 C 51

4.4.9Ascus and Ascospores 52

4.4.10. Myecelium type 53

4.4.11. Pellicle formation 54

4.4.12. Budding type 55

4.4.13. Colony morphology 56

4.4.14. Sugar fermentation 57

4.4.15. Sugar assimilation 58

4.4.16. Ethanol Tolerance 59

4.4.17. Amylolytic activity 58

4.4.18. Pathogenic contaminants 60

4.5. Biochemical analysis of *Haria* 62-64

4.6. Yeast characterizations. 65

4.7. Lactic Acid Bacteria 92-93

5.0 Photographs. 94-107

6. DISCUSSION 108-116

7. CONCLUSION 117

8. SUMMARY 118

9. BIBLIOGRAPHY 119-132

INTRODUCTION

One of the biggest challenges of today's world is food security for all the human beings. Many countries are facing food scarcity and many people across the globe are suffereing from malnutrition. In recent years we have noticed tons and tons of food grains get spoiled due to lack of storage facilities.Ancient people invented traditional food preservation technologies including fermentation, smoking, drying, and salting processes to preserve available plant or animal food sources and developed the culinary skills to make food recipes based on several biological, geographical and physical factors as well as individual sensory likings (Tamang and Samuel, 2010). Among them one of the most economically and oldest method for preserving and producing food is "FERMENTATION". The word "fermentation" is basically being derived from the Latin word "FERVERE" meaning "*to boil*". This phenomenon was observed during the production of alcoholic beverages where appearance of bubbles in growth medium was observed due to action of yeasts on growh medium.

Fermented foods are not only easy to preserve but also have benefits like it increases digestibility,pharmacological values, enhancing flavour and improving nutritional values.Unique group of micro flora related with each fermented food increases the levels of essential amino acids, vitamins, proteins and many more. Thus, $3/4^{th}$ of humanity which is facing a shortage of balanced food and endemic malnutrition, fermented food can play a vital role in fulfilling the diet requirement of world (Steinkraus, 1997; Tamang *et al.*, 2009).

Fermented foods are prepared from the raw ingredients of the plant or animal sources either by naturally or by mixing the starter culture(s) containing main functional microflora which is responsible for modification of the substrates biochemically and also organoleptically into edible yields that are socially and culturally acceptable to the consumers (Tamang, 2010 a,b).

Traditional fermentation is a form of food processing, where microbes, for example, lactic acid bacteria (LAB) and yeasts are utilized for food preservation. The bacteria use food as a substrate for their propagation and in the process produce substances which inhibit the growth of other harmful bacteria and enhance the nutritional value of foods. This form of food preservation technologies was used from ancient times and forms, which is part of the cultural and traditional norms among the indigenous

communities all around the world especially in Africa. The rural folk have come to prefer fermented over the unfermented foods because of their pleasant taste, texture and colour.This popularity has made fermented foods one of the main dietary components of the developing world (Aderiye *et al.*, 2003; Mosha *et al.*, 2004; Nout *et al.*, 1999).

Fermentation is one of the oldest and most economical methods for preserving foods and in addition to preservation, fermented foods have the added benefits of enhancing flavour, increased digestibility, improving nutritional and pharmacological values (Hesseltine, 1983). All the fermented food is associated with specific micro-biota which increases proteins, vitamins, amino acids and fatty acids in the foods. These fermented foods and drinks are good alternative of foods generally consumed by people (Jeyaram *et al.*, 2008).

Fermented foods are generally produced from plant or animal-based raw materials in combination with fungi or bacteria, which are either present in thenatural environment, or added intentionally by human to obtain the desirable end products (Law *et al.*, 2011). Availability of raw materials brings about the conversion of the raw materials to different form of fermented food products in order to increase the food varieties as well as to maintain food security (Nout *et al.*, 1997). (Berger *et al.* 1999) suggest that people who consume as little as one alcoholic drink per day may significantly reduce their risk of stroke, but drinking more does not increase the benefit.Tongnual and Fields, 1979 reported that lactic acid fermented rice improves nutritional value and available lysine content in rice. Ethnic, mild-alcoholic beverages from the Himalayas such as *kodo ko jaanr/chyang*, *bhaati jaanr*, *poko*, etc., have been consumed for therapeutic uses (Tamang, 2010).Among fermented foods, fermented beverages and alcoholic drinks are culturally and socially well accepted for consumption, drinking, entertainment, customary practices and religious purposes. Production and drinking of alcoholic beverages are widely accepted which not only enhancers the nutritional value but also gives pleasure of drinking (Darby, 1979). It is believed that Caucacians and Mesopotamian were using wine as early as 6000 B.C.Romans took it to Mediterranean and finally it reached India and china in 100 B.C(Robinson,1994; Pretorius,2000), however some reports suggest that alcoholic drinks were consumed in India during Ramayana (300 – 375 BC) and Vedic period (Prakash,1961).Platt (1964)referred to a traditional fermented beverageas the primary example of "biologicalennoblement" due to the bioenrichment with essential nutrients

through fermentation. Alcoholic beverages represent a vast diversity of products ranging from ethnic fermented beverages, alcoholic drinks, and distilled alcoholic products to wine and beer (Stewart, 1987). Ethnic, alcoholic beverages have strong ritualistic importance among the many ethnic communities of Asia. Ethnic alcoholic brewing is a home-based industry mostly practiced by rural women of Asia and Africa using their native skills of alcohol fermentation.The fermentation process to prepare alcoholic foods and beverages starts with hydrolysis of starch by amylolytic molds and yeasts, followed by ethanol or alcohol production by yeasts and flavor by lactic acid bacteria.Mycelial fungi present in fermented beveragesare mostly species of *Actinomucor*, *Amylomyces*,*Mucor*, and *Rhizopus* (Hesseltine1991; Lee and Lee, 2002; Nout and Aidoo, 2002; Samson and Hoekstra,2002). Yeasts associated with fermented beverages are species of *Saccharomyces*, *Saccharomycopsis*, *Schizo saccharomyces*, *Pichia*, *Hansenula*, *Candida*, *Kluyveromyces*,*Debaryomyces*,*Torulopsis*, and *Zygosaccharomyces* (Pretorius,2000; Tamang and Fleet,2009).Major yeasts, which ferment saccharified cereal starch to alcohol are, *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae* and *Candida lactosa* (Dung *et al.*,2005).Species of *Pediococcus* and *Lactobacillus* are frequently found in some ethnic amylolytic starters and fermented beverages (Tamang *et al.*,2007).Yeasts play most important role in preparation of alcoholic drinks like f beer and wine and strains of yeast which can rapidly and efficiently ferment sugar into ethanol and have ability to tolerate an ethanol concentration in the range of 15%–20% v/v are moref preferred for fermentation (Kodama,1993).Unique strains of *S. cerevisiae*have evolved to conduct these fermentations, generating products with high ethanol content (12%–20%), attractive flavors and aroma (Dung *et al.*, 2005, 2006). Cereal wine (*jaanr*) and distilled wine (*raksi*) in Sikkim is prepared and consumed (Kozaki *et al.*, 2000) Foods and alcoholic beverages produced by the process of fermentation with the help of yeasts and molds are generally regarded as safe foods and beverages which also include rice wine (Steinkraus, 1997). Rice beverage preparation involves alcoholic fermentation using mainly yeast from *Saccharomycopsis* sp. *S. fibuligera* with high ethanol producing capacity and amylolytic activities.*Saccharomycopsis* spp. is the common yeast present in rice wine starter in Southeast Asianregion (Limtong *et al.*, 2002).Yeasts dominate the microbial composition of many fermented beverages and few fermented foods of the world (Tamang and Fleet, 2009).Bhaanti Jaanr and kodo ko Jaanr are rice fermented drinks of Sikkim and Darjeeling region having

microorganisms like yeast, molds and lactic acid bacteria (Tamang *et al*, 2006; Thapa *et al.*, 2004). *Tapuy* is a sweet and acidic alcoholic rice wine whereas *ruou nep*is a turbid suspension of pink red color with some residual sugar and contained 8–14% (w/v) alcohol (Aidoo *et al.*, 2006).Traditional alcoholic brewing is a home based industry mostly practiced by rural women using their indigenous knowledge (Tamang, 2001).Different types of plants and their leaves are used in the starter culture preparation (Deka *et al.*, 2010, Deori *et al.*, 2007). Some of these alcoholic beverages are also used as drugs (Singh and Singh, 2006) which.Sujen is arice beer of Assam and is locally prepared by using yeast cakes (Deori *et al.*, 2007).It is reported also reported that rice beer being used as a drug (Singh and Singh, 2006). It works effectively against headache, body ache, insomnia, and inflammation of body parts, diarrhoea and urinary problems, expelling worms and as a treatment of cholera (Deka and Sarma, 2010). *Yu* is traditional alcoholic beverage of Meitei communities of Manipur. The yeasts isolated from Marcha, a starter culture showed ethanol production mainly by the isolated strains *S. bayanus, C. glabrata* and *P. anomala* (Tamang and Sarkar, 1995).The ethnic people of North East have developed the the ethnic foods to adapt to harsh conditions and environments for centuries (Tamang *et al.*, 2010).

Haria is one of the popular traditional rice fermented alcoholic beverage prepared by the tribal people of West Bengal such as Adivasi, Rava, Santhal, Saibo and Uraon. Preparation of *Haria* requires specific starter culture which is locally called *dabai* by the tribal people. *Haria* is prepared and consumed throughout the year.One of the most unique features of this tribe community is that only women are engaged in the *Haria* and *Dabai* preparation and its sale.Fermented foods play a significant socio-economic role in the developing world and these kinds of fermented food products contributes to the nutritional requirements of growing population.Fermented foods have several benefiting properties like it increases protein content, vitamins, amino acids and fatty acids in the foods along with its storage, preservation and inhibition of growth of many pathogenic bacteria.However, the knowledge of the fermented foods and beverages like "*Haria*" and "*Dabai*" preparation is getting eroded fast in younger generation due to changing lifestyle and availability of chemically preserved cheap and commercial foods. If the same trend will continue then this kind of traditional knowledge and practices will vanish very soon.Therefore, it is very important to document and preserve this kind of traditional knowledge,practices and conduct

research to standardize the procedure to prepare these kinds of fermented foods.Various indigenous communities uses fermentation process for the production of food items consumed by many people and therefore requires scientific studies as it has direct effect on human health.To best of our knowledge no previous studies have been done on Microbiology and Biochemistry of this product and on fermentation process and therefore require immediate attention and scientific studies.

Rationale of the study:

The present research work on *Haria* will be helpful to explore and document the indigenous knowledge of the tribal population and for preservation of traditional food culture.This kind of research will also help to create a reference database for future generations for food research scientists,nutritionist and food regulatory bodies and policy makers in the different ladders of the government.

Objectives of the research work:

1. Documentation of traditional knowledge of *Haria* and *Dabai* preparation by tribal people ofWest Bengal.
2. Enumeration of yeasts, moulds, lactic acid bacteria and spore formers in *Haria* and *Dabai.*
3. Isolation and characterization of yeasts from *Haria* and *Dabai.*
4. Determination of important properties of yeasts isolates of *Dabai* and *Haria.*

a) To study ethanol tolerance capacity of the yeast isolates of *Haria* and *Daba.*
b) To study ethanol content of *Haria.*
c) Determination of amylolytic activity of the yeast isolates of *Haria* and *Dabai.*

5. Characterization of pathogenic contaminations of *Haria* and *Dabai.*

REVIEW OF LITERATURE

Haria is an inexpensive alcoholic beverage prepared from steamed glutinous rice (arba-chawal) and consumed as food beverage in West Bengal. Tribes use their traditional knowledge to prepare Haria by fermenting the glutinous rice (personal communication). Traditional alcoholic brewing is a home based industry mostly practiced by rural women using their indigenous knowledge (Tamang, 2001).The fermentation of glucose to ethanol represents a series of coordinated enzymatic reactions. This process is internally balancing and thermodynamically favorable providing that cellular enzymes consume the net ATP generated from substrate-level phosphorylation *Saccharomyces cerevisiae* is used extensively in batch fermentations to convert sugars to ethanol for the production of beverages (Dombek *et al.*, 1987). Some of the well known alcoholic beverages are:

Atingba

Atingba is one of the famous traditional fermented alcoholic beverages of Manipur, prepared from rice. It is consumed by the Meitei community as food beverage on the various occasions.For *atingba* preparation, rice is cooked first and its watere is allowed to remove and then cooled to room temperature.The cooked rice is mixed properly with crushed *Hamei* (starter culture for Atingba) at the rate of 5 cakes/10 kg of rice. The mixed mass is then placed within earthen pots which is covered with leaves of *hangla (Alocasia* sp.) and is allowed to ferment for 3–4 days in summer and 6–7 days in winter season. This is then followed by 2–3 days of submerged fermentation in earthen container to give the final product alcoholic *atingba.* It is further distilled to give a clear-liquor alcoholic beverage called *yu* in the Manipur, India. There are different types of yeasts and Moulds microflora that are responsible for fermentation of rice to Atingba yeasts, *C. parapsilosis, C. montana, Saccharomyces cerevisiae,Pichia anomala, P. guilliermondii, P. fabianii, Trichosporon* sp., *Candida* are *Mucor* spp. and *Rhizopus* spp; *tropicalis* and *Torulaspora delbrueckii*; whereas some important LAB are *Lactobacillus brevis ,Pediococcus pentosaceus* and these microorganisms are playing important role in flavour and texture development (Tamang *et al.,* 2007; Jeyaram *et al.*, 2008).

Bhaati Jaanr

One of the popular Jaanr prepared and consumed in Darjeeling and Sikkim hills is *Bhaati Jaanr*.It is traditional fermented mild alcoholic beverage of these regions.It is prepared by using glutinous rice, and marcha is used to ferment the rice, first rice is sccharified for 1-2 days in an earthen pot at room temperature and once the saccharification is completed vessel is made airtight and is kept for formentation for 2-3 days in summer and 7-8 days in winter.The main micro-organisms which are involved in *Bhatti Jaanr* saccharification and fermentation are Filamentous molds(*Mucor circinelloides*, *M. hiemalis*, *R. stolonifer*,*Rhizopus chinensis*, and var. *lyococcus*) and yeasts (*Saccharomyces cerevisiae*, *S. bayanus*, and *Candida glabrata)*, and Lactic acid bacteria like(*Pediococcus pentosaceus*, *Lactobacillusbifermentans*, and *Lb. brevis)* (Tamang and Thapa, 2006).These microflora is responsiblec for the flavour development and acidity of the product.The PH,titrable acidity , moisture content and ethanol content of the of the Bhaati jaanr is 3.5, 0.24%, 83.4% and 5.9%. *Bhaati jaanr* is consumed as a food directly (Tamang, 2010).

Makai ko Jaanr

Some places of Sikkim and Darjeeling hilly regions are mainly envolved in the Makai *ko jaanr* preparation and consumption,itis a viscous, slightly bitter, mild alcoholic beverage .It is prepared by fermenting the substrate maize.The microflora that are responsible for fermentation of the Makai ko jaanr are mainly Filamentous molds, *Rhizopus chinensis*,*Mucor circinelloides*, *M. hiemalis*,and *R. stolonifer* var. *lyococcus*; yeasts sp.involved in fermentation are .*Saccharomycopsis fibuligera*, *Pichia anomala*,*P. burtonii*, *Saccharomyces cerevisiae*,*Candida glabrata*; lactic acid bacteria *Pediococcus pentosaceus*, *Lactobacillus bifermentans*, and *Lb. brevis*.These microflora gives *makai ko Jaanr* a special flavour and test .Marcha is again used as a starter culture for the *Makai ko Jaanr* fermentation (Thapa and Tamang, 2001). It is also prepared and consumed in some parts of Nepal (Thapa and Tamang, 2006).

Kodo ko Jaanr

Kodo ko jaanr is a popular fermented alcoholic beverage prepared from dry seeds of finger millet (*Eleusine coracana*), which is locally known as *kodo* in Darjeeling hills, Nepal, and Sikkim (Tamang *et al.*, 1996). Seeds of finger millet are cleaned, washed, and then cooked for about 30 mins in an open cooker.After cooking excess of water is

drained off,and cooked millets are spread on a clean bamboo mat, locally known as *mandro*, for cooling. About 1%–2% of powdered *marcha* is starter culture for *Kodo ko jaanr* is sprinkled over the cooked seeds, and then mixed well, and mixture is packed in a bamboo basket lined with fresh fern, locally known as *thadre uneu* (*Thelypteris erubescens*), or banana leaves.It is then covered with sackcloth, and is kept for 2–4 days at room temperature for saccharification.During the process known as saccharification, a sweet aroma is then emitted, and the saccharified mass is transferred into an earthen vessel. The cooked finger millet seeds are fermented for 3–4 days in summer and 5–7 days during winter at room temperature. Good-quality *Kodo koJaanr*has a sweet taste with a mild alcoholic flavour. Prolonged fermentation makes the Jaanr bitter in taste and more alcoholic.Consumers generally reject *kodo kojaanr* with sour taste and unpleasant flavor (Thapa *et al.*, 2004).Microorganisms responsible for fermentation of kodo ko jaanr are Filamentous molds, like *Mucor circinelloides* and *Rhizopus chinensis*; yeast microflora involve in fermentation are *Saccharomycopsis fibuligera, Saccharomycopsiscapsularis, Pichia anomala, P.burtonii, Pichiaanomala,Saccharomyces cerevisiae,Candida glabrata*,and *Saccharomycopsis fibuligera* and lactic acid bacteria playing important role in fermentation are *Pediococcus pentosaceus* and *Lactobacillus bifermentans* (Tamang *et al.*, 2007; Jeyaram *et al.*, 2008).

Gahoon ko jaanr

This is one of popular traditional alcoholic beverage of Sikkim and Darjeeling hills.Gahoon*ko jaanr* is an mild alcoholic beverage prepared from wheat fermentation by using marcha as starter culture. The traditional method of preparation of *gahoon ko jaanr* is almost same as that used for *kodo ko jaanr*.This alcoholic beverage is consumed directly after filtering the fermented grits.Sometimes,*Gahoon ko jaanr* is mixed with *kodo ko jaanr* and filled up in a special container known as, *toongbaa. Gahoon ko jaanr* is mostly used for the distillation process to get *raksi,* a clear, distilled liquor.The main Microflora thatresponsible for fermentation of wheat are, yeast, LAB,moulds. The major yeast flora responsible for the fermentation are*Saccharomycopsis fibuligera, Pichia anomala, P. burtonii, Saccharomyces cerevisiae*, and *Candida glabrata*; filamentous molds(*M. hiemalis, Rhizopus chinensis,Mucor circinelloides*, and *R. stolonifer* var. *lyococcus*). Lactic Acid Bacteria

mainly,and *Lactobacillus bifermentans and Pediococcus pentosaceus*(Thapa *et al.*,2001).

Chhang

Chyang is prepared from a barley (*Hordeum nulum*) locally known as *sherokh* in Ladakh (Bhatia *et al.*, 1977). *Chyang* or *lugri* is a mild, foramy and translucent mild alcoholic beverage, which is prepared by fermentation.This fermented alcoholic beverage having a sweet-sour taste and aromatic flavour (Batra and Millner, 1976; Batra, 1986). In chhang preparation, first Barley grains are cooked over a slow fire in the water just sufficient for absorption and just after cooking this is spread on blanket or a burlap mat to remove the aceess of free water. The cooked barley grains at lukewarm stage are inoculated with *phab* using 1g/kg of barley.These contents are filled in drill bags,most oftenly in 20-kg batches, and tightly packed .These mass are further wrapped by gunny bags to maintain the temperature around 30°C–35°C which is important for the ferermentation of the barley and placed it to ferment for 7–8 days (Bhatia *et al.*,1977).Microflora that play impotant role in the fermentationprocess of Chhang areYeasts, *S. uvarum Saccharomyces cerevisiae* (Batra, 1986).This is one of the popular mild alcoholic beverage prepared and consumed by the people of of the Ladakh (Bhatia *et al.*, 1977).

Sujen

Sujenis an alcoholic beverage is popular among the Deori tribe of Assam. It is also considered to be pure and used as a holy water by the Deoro priests during various festivals and ceremonies.*Sujen* is prepared in two stageps, first is the preparation of of the natural starter called mod pitha and then the brewing of the Sujen (Deori *et al.*, 2007).For preparation of the mod pitha a variety of plants species used.Three to five kg of saol (rice) is soaked in water for about 2 hours and mixed with the dried plant matrials in a grounded in a wooden grinder called dheki along with two to three mod pithas.The grounded powder is sieved in asaloni and the fine powder is taken in a metallic vessel for a period of 7-15 days.Once the fermentation is completed it is diluted with water and and filter it for consumption (Das *et al.*, 2012).

Jann

Jann is traditional soft drinks of Bhotiyas,and contains very loe concentrations of alcohol.During *Jann*preparation,rice is cooked for about half an our then excess of water is removed from cooked rice and isspreaded on a flat container following to becooled quickly.Then *balam* powder is sprinkled over the cooked rice and mixed properly. The mixture is then kept in an airtight container (the mouth of the cloth container is sealed with a piece of cloth). And kept in a dark room for the fermentation. Generally in cold conditions the fermentation is slow than the warm condition.After a week of fermentation the product is ready for consumption (Roy *et al.*, 2004).

Lao-Chao

It is one of the famous alcoholic fermented beverages of China.In the preparation of Lao-Chao, rice is boiled first and then spread on a mat for cooling, and then it is mixed properly with yeast grown on rice and nosan leave.The inoculated rice is then poured into a cone-shaped bamboo basket and an earthenware pot or vessel is placed under the cone to collect the liquified rice as it ferments.The fermented juice is collected and transferred to new boiled rice about 3 or 4 times in succession.The major microflora that is responsible for fermentation of the rice consists of moulds mainly *Mucor, Rhizopus* and as well as yeasts and lactic acid bacteria (LAB).These microflora were playing the significant role in product. Development.The Final ethanol content of the product ranges from 12 to 14% (v/v). The pH of the product fell down from 6.7 to 3.9 (168 h). The ethanol content of this alcoholic beverage reached a level of 7.8% v/v (168 h) using the combination of *Hansenula* sp, *R. arrhizus* and reducing sugars of the product reached concentrations as high as 7.2 to 10.0% (168 h). (Wang and Hesseltine, 1970; Wei and Jong, 1983).

Poko

Poko is one of the famous traditional rice fermented alcoholic beverage of Nepal (Shrestha *et al.*, 2002). It is similar to the fermented alcoholic beverage *Bhatii ko Jaanr* of Sikkim and Darjeeling.It is commonly consumed and served during the festive seasons by the people of Nepal. The major microflora that playing significant role in fermentation of Product are mainly *Rhizopus* as well yeasts play significant

rolein the fermentation and product development,like *Candida versatile, Saccharomyces cerevisiae* and lactic acid bacteria (LAB), *Pediococcus pentosaceus also playing* important role in the product development.This is an important traditional alcoholic beverage of Nepal have strong socio-cultural significance (Shrestha *et al.*, 2002).

Tapé ketan

It is a sweet/sour rice fermented alcoholic beverage of Indonesia.The most common substrate used for fermentation is cassava (tapé ketella) followed by glutinous rice (tapé ketan). For the preparationof *Tapé ketan* the Glutinous rice is washed, soaked for 1 h or longer in water, steamed until cooked well, spread over a woven bamboo tray, and is allowed to cool to room temperature.Then powdered ragi (starter culture) is sprinkled over the rice, and then mix properly and is placed in an earthenware pot, an enameled pan, or a glass vessel to ferment. Within 2 to 3 days at room temperature, the sticky rice is converted to a soft, juicy mass with a sweet/sour; alcoholic flavor which is ready for consumption.The product is acceptable even after 6 or more days of fermentation. (Cronk *et al.*, 1977).With the continued incubation the product becomes more liquid. Tapé ketan gets ready for consumption after 36 to 48 h of fermentation at 30°C and by 96 hours nearly all significant changes in the product taken place.Beyond this time the changes are slight and the product remains edible (Cronk *et al.*, 1977). Malaysian tapai is also alcoholic beverage contains of 23% of reducing sugar, 27% of total sugar, 5% of ethanol (v/v), and its pH is 3.9 is acidic in nature.Tapé ketan must be sweet to be acceptable therefore, the final product should be consumed between 72 and 96 hours when reducing sugars of the product are highest (Merican and Yeoh, 1977).

Ruhi

Ruhi is a stronge alcoholic beverage prepared by fermentation of boiled rice.It has some similarity with Indonesian *tapé ketan* but differs in its method of collection of the alcoholic liquor. *Ruhi* is one of the social drinks primarily prepared by tribal peoples in Nagaland and in the North Eastern part of India.Ii has a strong socio-cultural importance among the tribes of Nagalan.The major microflora responsible for *Ruhi* fermentation consists of molds of genera mainly *Rhizopus* and *Mucor*, as well as yeasts and lactic acid bacteria also playing an important role in fermentation and

product development.The final ethanol content of the product ranges from 12 to 14% (v/v). The pH of the *Ruhi* is about 4.0. The Reducing sugars of the final product are 2.5 % (w/v) with total sugar being 3.0% (w/v) (Dahiya and Prabhu, 1977).

Sake

Sake is a rice fermented alcoholic beverage and national drink of Japan.It is highly popular in japan and is declared as national drink of Japan.It is a clear, pale yellow rice beverage with an alcoholic content of 15 to 16% or higher, little acid, with a characteristic aroma and slight sweetness (Murakami, 1972). Japanese sake is mostly related to Chinese rice wine, *shaosing chu.*Both Sake and *Shaosing Chu* preparation requires a *koji* to saccharify the starch of the rice.Shaosing chu has a deeper color and a more oxidized cherry like flavor. The preparation of sake includes various steps.Firstly boiled rice is allowed to ferment and is used as inocula and is mixed with freshly boiled rice and water. After some days, it fermented and product is formed is consumed.Saccharification and fermentation proceed simultaneously in the unfiltered, dense, mushy mesh (called *moromi).* This particular set of circumstances leads to very higher populations of yeasts in the mash and high ethanol contents concentration, nearly 20% (v/v) in the sake.About 280 kilo liters of sake can be prepared from 150 tons of brown rice (Kodama and Yoshizawa, 1977). Protein content of the product is, low but sake contains 18 different amino acids in amounts ranging from traces to few milligrams, and for example sake contains uoto 3 mg tryptophan/L and 53 to 105 mg leucine/L (Kodama and Yoshizawa, 1977). Sake is one of the sources of income for Japanese government; they are earrning good amount of revenue from this fermented alcoholic beverage.

Tapuy

Tapuy (Igorot) is a highly acidic but sweet, aromatic, alcoholic rice beverage whose pleasantly balanced flavor makes it a substitute for distilled liquors among native people of certain regions of the Philippines.It is known by other names as *binubudan* (Ifugao), *binuburan* (Ilocano),or *purad* (Tagalog).During the process of preparation of Tapuy, Glutinous rice or ordinary white rice or a mixture of the two is washed well and then ground in a stone mill. The mash is mixed with pureed ginger and/or wild unwad root,the starter culture is shaped into small balls or flattened discs, inoculated

with powdered bubod from previous batches, incubated for 3 days, and dried (Sakai and Caldo,1983).*Saccharomyces uvarum* and *Saccharomycopsis* are the dominant yeast flora playing important role in the fermentation of the tapuy (Sakai and Caldo,1985).(Sakai and Caldo,1985) ,were reported that glucoamylases were the primary amylases produced by *S. fibuliger, S. burtonii,* and *Mucor* important moulds playing significance role in the fermentation and product development.Ethanol concentration of the product is 4.93%(v/v) day 2 and reached a level of up to 15.5% v/v (day 14)(Sanchez *et al.*,1985) reported that eight different bubod,starter cultures yielded ethanol contents of 12.9 to 17.3% (v/v) in tapuy.PH of the final product ranged between 3.96 to 4.49.

Yakju and Takju

The starter for yakju and takju is called *nuruk.* Presently, the substrate for nuruk is wheat, but historically it was rice. The starter culture is generally prepared by natural inoculation of moulds, bacteria, and yeasts; however, it can be prepared by inoculation with *Aspergillus usamii.*Moistened wheat flour ferments spontaneously and is ready to use in 2 to 3 months. Similar to a wheat-based koji, nuruk serves as a source of amylases for the conversion of starch to sugar, and then sugar is converted in to ethanol by *S. cerevisiae,* significant yeastplying important role in sugar fermentation.

The liquid pressed from the fermenting mass is filtered under pressure, aged, and then bottled. When *A. usamii* wheat koji is used, a yeast inoculum is added along with the koji to moist steamed wheat flour (27 to 30% moisture). This mixture is then fermented, filtered, and aged as in the traditional process. Although saccharification and ethanol production occur simultaneously, most of the starch is broken down in the first 24 hours to monosaccharides sugars and sugar converted to ethanol.Thus, a quasi-two-stage fermentation takes place. In two samples of natural nuruk, (Kim ,1968) isolated, per gram of sample, 1.6×10^7 to 2.9×10^7 cfu of *A. niger,* 2.0×10^7 to 8.4×10^7 colony-forming units (cfu) of *A. oryzae,* 2.0×10^6 cfu of genus *Rhizopus,* 1.3×10^7 to 2.6×10^7 bacteria, 5.0×10^6 to 2.0×10^7 aerobic bacteria, and 1.4×10^5 to 6.0×10^5 yeasts (Kim, 1968).During yakju fermentation, moulds population were disappeared after 2 or 3 days of fermentation and aerobic bacterial population increase and then rapidly decrease with growth of lactic acid bacteria (LAB). *S cerevisiae* is most important yeast playing important role in alcohol production and *Hansenula* sp.microflora responsible for flavor development in the product (Kim and Lee, 1970).

Tchoukoutou

This is one of the many different traditional African beers.It is prepared from red sorghum. During the process of preparation of this alcoholic beverage, first sorghum grains are cleaned, soaked, and allowed to germinate (sprout) this is followed by a careful sun-drying in order to stabilize the obtained malt.After polishing and grinding the malt, it is mashed with water at a gradually increasing temperature until the final boiling, within a total period of 4–5 hours (Kayode *et al.*, 2007).The wort obtained is decanted, cooled, and is transferred to fermentation containers that contains active yeast sediment.There Extensive research has been done on this beverage and is showed yeasts is the main flora like, *S. cerevisiae*, helps in the fermentation process of *Tchoukoutou* .*Tchoukoutou* has a low (3%–4% v/v) ethanol content and this beverage locally regarded as a refreshing healthy beverage.In addition to the attractive sensory attributes, it was observed that the fermentation of *Tchoukoutou* results in very good availability of mineral like, iron (Kayode *et al.*,2007).

Zutho

This is one of the popular mild alcoholic beverages of the Mao of Naga community.For the preparation of zutho , first rice is soaked in water overnight, water is removed , grinded in to powder form and this is put in to bamboo bucket and mixed properly with boiling water , after cooling the amylolytic starter which is locally known as " Khekhrii" (Mao and Odyuo,2007) in the in the powdered form is mixed and kept about 6-8 hours for brewing .After mixing the whole mixture is poured in to earthen pot and more water is added up to neck .This pot is now kept for fermentation 3-4 days (Mao,1998).Nchiangne is the similar alcoholic beverage is prepared from rice in Nagaland(Tamang *et al.*,2012). The pH of *zutho* is about 3.6, and its alcohol contents is about 5% and acidity of the product is about 5.1% (Teramoto *et al.*,2002).

Marcha

The mostly frequently used starter culture in the Himalayan regions of India, Nepal, Bhutan, and Tibet in China is the Marcha (Tamang, 2010).*Marcha* is a inocula prepared as a dry, creamy white to dusty white, round to flattened, solid elliptical ranges from 1.9 to 11.8 cm in diameter.Is mainly prepared by the womens of Darjeeling and Sikkim regions, in their home. It is a home based industreis.Marcha is mainly used as an amylolytic starter to produce traditional fermented alcoholic

beverages in this hilly area.During *marcha* preparation, glutinous rice is washed soaked in water for 8–10 h, and then rice is crushed in a foot-driven heavy wooden mortar by a pestle. In powdered rice, various ingredients are mixed which include roots of *Plumbago zeylanica*, Leaves of *Buddleja asiatica*, ginger, red dry chilli, flowers of *Vernonia cinerea* and 1%–2% of previously prepared *marcha* usedas the mother culture. Round and flat cake is prepared by the mixed rice powder and the plants leaves. A specific alcoholic and ester flavour and swollen appearance of the *marcha* indicates the completion of fermentation process, and fresh cakes of *marcha* are sun dried for 2–3 days (Tamang *et al.*, 1996).*Marcha* is stored at a dry place at room temperature and it can be used up to one year for the Jaanr preparation.The population of moulds in *marcha* is About 10^6 cfu/g, the yeast and lactic acid bacteria (LAB) loads are in the range of 10^8 and 10^7cfu/g, respectively.The main moulds species present in *marcha* are *circinelloides*, *Mucor* close to *M. hiemalis* and *R. chinensis*, a *Mucor circinelloides* and *R. stolonifer* variety *lyococcus* (Tamang *et al.*, 1988).

Ragi

Ragi is an important amylolytic starter from Indonesia in the form of dry round and flat cakes (Saono *et al.*, 1974).During the production of *ragi*, rice or millet or cassava or other starchy bases are properly,mixed with herbs, plants with medicinal properties and spices, roasted together, and then sieved.The mixture is mixed with water and 2%–4% powder of old *ragi*, mixed thoroughly, it is prepared like balls, and is fermented at 25°C–30°C for 72 h in a humid environment.Fermented balls are sun dried and used as a starter culture for the preparation of alcoholic beverages and drinks in Indonesia. (Kato *et al.*, 1976) studied the properties of glucoamylase of *Saccharomycopsis fibuligera*isolated from *ragi*.

Bubod

Bubod is used as an ethnic amylolytic starter culture in the Philippines. During its preparation, rice and ginger are powdered, and mixed properly with enough water and make the round, flattened yeast cake (Tanimura *et al.*, 1977). The round balls are coated with 1–3 month old *bubod* and incubated in rice straw for 36 h at room temperature and then are sun dried. The major population of yeasts in *Bubod* about 10^7–10^8 cfu/g, filamentous moulds in *bubod* was about 10^3–10^5 cfu/g, and Lactic acid

bacteria (LAB) is about 10^5–10^7cfu/g (Sanchez, 1986).*Mucor circinelloides*, *Saccharomycopsis fibuligera Rhizopus cohnii*, *M.* and have been observed from *bubod* (Kozaki and Uchimura, 1990) However, *S. fibuligera* is main dominant amylolytic yeast flora in the starter culture bubod(Hesseltine and Kurtzman, 1990).The activated starter culture where used for production of *basi*, a mild alcoholic beverage consumed as a food in the Philippines, is known as *binubudan* (Tanimura *et al.*, 1978).

Nuruk

This is ethnic amylolytic starter from Korea. Historically, the substrate for *nuruk* was rice but at now it is wheat (Park *et al.*, 1977). Generally *nuruk* is made by natural inoculation of yeasts, moulds, and bacteria, and however, it can be prepared by inoculation with *Aspergillus usamii.* Traditionally, *nuruk* is prepared by moistening wheatflour, kneading and molding into different sized balls, and allowed for fermentation for 17 days at 30°C–45°C, then allow to dried for about two weeks, and then cured for 1–2 months at room temperature (Park *et al.* 1977).(Kim ,1968) isolated *A.niger* (107 cfu/g),*Aspergillus oryzae* (107 cfu/g),*Rhizopus* sp.(106 cfu/g),aerobic bacteria (106–107 cfu/g),anaerobic bacteria (107 cfu/g),and the yeasts loads are about (105 cfu/g) from *nuruk.*

Loogpang

Loogpang is an ethnic amylolytic starter from Thailand, which is commonly used to prepare alcoholic drinks and vinegar.The main ingredient of *loogpang* is rice flour mixed with different types of spices and microorganisms.The microorganisms originate from the inoculum, from the surroundings, or from the previous batch (Vachanavinich *et al.*, 1994). Species of moulds present in *loogpang* are *Amylomyces*, *Rhizopus*, *Aspergillus*, *Mucor*, and *Absidia* (Pichyangkura and Kulprecha, 1977), and yeasts and LAB are *Saccharomycopsis fibuligera*, *Hansenula*, *Saccharomyces*, and *Pediococcus* (Dhamcharee, 1982), (Uchimura *et al.*, 1991).*Sm.fibuligera* isolated from *loogpang* has high glucoamylase activity (Sukhumavasi *et al.*, 1975).

Men* or *Banh

Men or *banh men* is an ethnic amylolytic starter from Vietnam which is used for producing traditional alcoholic beverages and a drink called *ruou* (Dung, 2004).

During the preparation of *men*, uncooked rice flour is mixed with local herbs and spices and moistened with a little amount of water to form dough, which is then made into small balls or flattened discs. The dough is spread on a bamboo tray and mixed with previously prepared powdered *men*. The inoculated dough discs are incubated at room temperature for a few days. Moulds such as *Amylomyces rouxii* and *Rhizopus*, yeasts such as *Hyphopichia burtonii*, and *Saccharomyces cerevisiae*, *Saccharomycopsis fibuliger*,and Lactic Acid Bacteria wereobserved in*men* (Dung *et al.*, 2006, 2007). *S bayanus* has not been isolated from *men*, but the closely related *S. cerevisiae* has been isolated from *banh men* (Lee and Fujio,1999).On the basis of a PCR-mediated DGGE system, several yeasts and LAB have been isolated from *banh men* that consist of amylase producers (*Rhizopus oryzae*, *R. microsporus*, *Absidia corymbifera*, *Amylomyces* sp., and *Saccharomycopsis fibuligera*), ethanol producer (*Saccharomyces cerevisiae*, *Issatchenkia* sp., *Candida tropicalis*, *Pichia anomala*, *P. ranongensis*, and *Clavispora lusitaniae*), yeast contaminants (*Botryobasidium subcoronatum*,*Xeromyces bisporus*, and), LAB (*pentosaceus*,*Lactobacillus plantarum*, *Lb. brevis*, *PediococcusWeissella confusa*, and *W. paramesenteroides*),amylase-producing bacilli (*Bacillus subtilis*, *B. circulans*, *B. amyloliquefaciens*, and *B.sporothermodurans*),acetic acid bacteria (*Acetobacter orientalis*, and *A. pasteurianus*),and environmental contaminants (*Burkholderia ubonensis*, *Ralstonia solanacearum*,and *Pelomonas puraquae*) (Thanh *et al.*, 2008).

Chiu-Yueh

Chiu-yueh is a Chinese amylolytic starter which is used for *lao-chao* preparation, a fermented rice beverage of China(Wang and Hesseltine, 1970).It is a white-grey ball containing dominant flora of yeasts and fungi grown on rice flour and is closely related to the Indonesian *ragi*. *Rhizopus*, *Amylomyces*, *Torulopsis*, and *Hansenula* species are present in *chiu-yueh* (Wei and Jong, 1983).

Hamei

Hamei is a traditional amylolytic mixed dry,round or flattened starter from Manipur, India, which is very almlost similar to *marcha* (Tamang, 2010). The prepared yeast cakes are kept over paddy husk in a bamboo basket, and then covered by sackcloth for 2–3 days at (20°C–30°C), room temperature and then this is sun dried for 2–3 days. The principle Yeast flora present in *hamei* is *Saccharomyces cerevisiae*, *Pichia*

anomala, and *Trichosporon* (Jeyaram *et al.*, 2008).The dominant (LAB) microflora is*Pediococcus pentosaceus* and *Lactobacillus plantarum* are present in *hamei* (Tamang *et al.*, 2007).

Mana

It is one of the important traditional starter cultures of Nepal.*Mana* is a granular-type starter prepared from wheat flakes (Tamang, 2010).During its preparation, wheat grains are soaked in water overnight, then steamed for 30 min and then transferred to a bamboo basket for removal of water and ground into a lump.On the clean floor straw is sprinkled and wheat lump is kept over it after that it is covered with paddy straw mat, and then it is allowed to ferment for the 6–7 days.After 7 days, when green filamentous fungi (moulds) appears on the wheat grains then, it is dried in the sunlight to get *mana* and is stored for further use.*Mana* contains about 10^6cfu/g of mucorales,10^3 cfu/g of yeasts,10^7 cfu/g of *aspergillilus*,and 10^5 cfu/g of Lactic Acid Bacteria (Nikkuni *et al.*,1996) . *Aspergillus oryzae* and *Rhizopus* spp. are the dominant species present in *mana* (Shrestha *et al.*, 2002).

METHODS AND MATERIAL

3. METHOD AND MATERIAL

3.1. CULTURE MEDIA USED

(1) Casein enzymic hydrolysate (RM014, Hi Media, Mumbai)

(2). Ascospore Agar (M804, HiMedia, Mumbai)

(3). *Bacillus cereus* Agar Base (M833, HiMedia, Mumbai)

(4). SS Agar (Himedia, Mumbai)

(5). Baird Parker Agar Base (M043, HiMedia, Mumbai)

(6). Egg Yolk Emulsion (FD045, HiMedia, Mumbai)

(7). Egg Yolk Tellurite Emulsion (FD046, HiMedia, Mumbai)

(8). Fermentation Basal Medium for yeasts (Wickerham, 1951)

(9). *Listeria* Identification Agar Base (PALCAM) (M 1064, HiMedia, Mumbai)

(10) Malt Extract Agar (M137, HiMedia, Mumbai)

(11). MRS Agar (M641, HiMedia, Mumbai)

(12). MRS Broth (M369, HiMedia, Mumbai)

(13). Nitrate Broth (Gordon *et al.*, 1973)

(14). Nutrient Agar (MM012, HiMedia, Mumbai)

(15). Nutrient Broth (M002, HiMedia, Mumbai)

(16) Plate Count Agar (M091, HiMedia, Mumbai)

(17). Potato Dextrose Agar (M096, HiMedia, Mumbai)

(18). *Salmonella–Shigella* Agar (M108, HiMedia, Mumbai)

(19). Skim Milk Powder (RM1254, HiMedia, Mumbai)

(20). Sucrose Broth (Garvie, 1960)

(21). Tryptone Soya Agar (M290, HiMedia, Mumbai)

(22). Violet Red Bile Glucose Agar w/o Lactose (M581, HiMedia, Mumbai)

(23). Yeast-Malt Extract (YM) Agar (M424, HiMedia, Mumbai)

(24). Yeast Malt Extract (YM) Broth (M425, HiMedia, Mumbai)

(25) .Streptomycin sulphate and Benzylpenicilline (HiMedia, Mumbai).

(26). Yeast Morphology Agar (M138, HiMedia, Mumbai)

(27). Yeast Nitrogen Base (M139, HiMedia, Mumbai)

3.2. Reagents used

(1). Acidic Ninhydrin

1-Butanol/water saturated	465 ml
Acetic acid	35 ml
Ninhydrin	2.5 ml

2). Lugals Iodine Solution

Iodine	2.0 g
Ammonium Sulphate	2.0 g
Distilled water	300 ml

(3). Gram's Crystal Violet (S012, HiMedia, Mumbai)

(4). Malachite Green (S020, HiMedia, Mumbai)

(5). Nessler's Reagent

Potassium iodide	50.0 g
Mercuric chloride (saturated)	35.0 ml
Distilled water (ammonia free)	25.0 ml
Potassium hydroxide (50 %)	400.0 ml

Potassium iodide was dissolved in 35 ml of distilled water followed by addition of saturated aqueous solution of mercuric chloride till the appearance of precipitate. Then, 400 ml of potassium hydroxide was added and made the final volume to 1000 ml by adding distilled water. The solution was left for a week; the supernatant was decanted and stored in capped amber bottle at 4° C

(6). Nitrate Reduction Test Reagent

Solution A

Sulphanilic acid	0.8 g
5 N acetic acid	100 ml
(Glacial acetic acid: water, 1: 2.5)	

Solution B

∝-Napthylamine	0.5 g
5 N acetic acid	100 ml

The solutions A and B were mixed in equal quantities just before use.

(7). Phenolphthalein (I009, HiMedia, Mumbai)

(8). Safranin (S027, HiMedia, Mumbai)

(9) Methyl red (Himedia)

(10) oxalic acid (MLOM, Merk Mumbai).

(11) Ethanol (Bengal Chemical, Calcutta).

(12) (0.05N) Sodium Hydroxide Solution (NAOH) (MF8D, Merk, Mumbai).

3.3. METHODS

3.3.1. Survey and documentation of indigenous, traditional knowledge of *Haria* and *Dabai* preparation:

The survey was done in different parts of North Bengal like Matigara, Shivmandir, Chandmuni, Champasari, Milanmore and Malbazzar to document the traditional indigenous knowledge of preparation of *Haria*, its mode of consumption, per capita consumption, socio-economic importance, its marketing and its ethnic significance.Detailed informations of *Haria* preparation were collected by interviewing different group of indigenous ethnic people of the North Bengal and its surrounding areas.This knowledge also includes indigenous,traditional method of preparation of *Dabai*,a kind of yeast cake that is acts as starter culture for *Haria* fermentation,its bio-preservation, and mode of use of *Dabai*,its socio-economic importance,its marketing and ethnic significance.Documentation of indigenous knowledge is important to preserve the traditional knowledge of preparation of fermented foods by of the ethnic community of West Bengal.

To document the traditional knowledge of preparation of fermented food *Haria* and *Dabai* seven different places as well local market of North Bengal have been visited, tribal people of different villages who are engaged in preparation of this fermented beverage were interveiwed,photographs of preparation method of *Haria* and *Dabai* were taken and traditional knowledge,socio-cultural importance of these fermented foods were documented.*Haria* is one of the popular traditional rice fermented alcoholic beverage prepared by the tribal people of West Bengal such as Adivasi, Rava, Santhal, Saibo and Uraon.Preparation of *Haria* requires specific starter culture which is called as *Dabai* by the local tribal people. *Haria* is prepared and consumed throughout the year.One of the most unique features of this tribe community is that only women are engaged in the *Haria* and *Dabai* preparation and its sale. *Haria* is an ethnic fermented rice beverage,consumed as a staple food beverage by the tribal community of West Bengal.The method of preparation of *Haria* includes various steps, first the Arba-chawal (Glutinous rice) is washed then Glutinous rice is cooked for about 20 min, drained off and 5% of powdered *dabai*,the starter culture is sprinkled over cooked rice, mixed well and placed in a container or in an earthen pot for 1-3days at room temperature for the saccharification of starch of rice the (Arba-

Chawal).Then, the container is made airtight and the saccharified rice was fermented for 2-3 days in a summer and 7-8 days in a winter. After fermentation water is added to the fermented mass, mixed properly and is filtered with a clean cotton bag and collected in a clean vessel, after which Haria gets ready for consumption.It is consumed directly as a food beverage.*Dabai* is a dry, round to flattened, solid ball-like mixed dough inoculum used as starter cultures to prepare *Haria* an alcoholic beverages of West Bengal. Local varieties of rice like Arba -chawal without soaking or soaked, and sundried, are crushed and mixed with crushed leaves of different plants,(Ghat patta)and a small amount of previously made powdered *Dabai*.The prepared dough is pressed into flat and round cakes and then placed over carpet and sundry for 3-5days in summer and 3-7 days in winter.After sundry the starter culture *Dabai* is kept with paddy husk in a bamboo basket, and covered by clean clothes and preserved at room temperature for further use.Mainly women are engaged in preparation and sell of *Dabai* in local markets of North Bengal.

3.3.2. Mode of consumption

It may be projected that 50–400 g per capita of fermented foods and alcoholic beverages are consumed daily worldwide (Tamang *et al.*, 2010).During survey in the tribal areas of North Bengal we found that *Haria* is consumed directly as a food beverage mainly by the people of tribal areas of West Bengal where, Adiwasi population is dominant it is also consumed by other population residing in these areas. Occasionally, *Haria* is stored in an Earthenware crock for 1-2 months which results in production of thick, white supernatant liquor, locally known as gadha *Haria* that might contains high amount of ethanol.It is served on some special occasions and festivals of ethnic tribal communities.*Haria* is not very expensive it is used to regain the strength and good health. *Haria* is sold in the local markets or hat in the villages. Although *Haria* is available in the markets, however some people prefer to prepare it athome for their own consumption.*Haria* is a mild alcoholic drink is manly consumed in liquid form after fermentation of rice.Depending upon the taste, varying amount of water people add to the fermented rice mass of *Haria*. From our surveys and interviews we found that the per capita consumption of *Haria*, in this part of North Bengal is 120g/day, which accounts for 10% of daily consumption of total food. *Haria* is integral part of the social life of these tribal communities and even guests are also

served with this drink.Normal five to seven days fermented *Haria* containing less amount of ethanolis served to children's on particular occasions. Before serving *Haria* to guests, the host test *Haria* for its flavour and aroma and then it is served to guests. It is a traditional way of consumption or it can be put in glasses and consumed otherwise.Men consumes it on social gatherings and traditional occasions but sometimes women consume it as a medicine. It can be consumed along with other eatables also.

3.3.3. Socio-economy and marketing data

Haria is prepared in most places of West Bengal as it is available in *Matigara, Chandmuni, Champasari, Shivmandir, Devidanga, Milanmore, and Malbazzar* and some other villages of North Bengal. It is not commercially available in the market like other beverages. One has to buy it from the houses where it is prepared and from the Hat of villages. Normally, people order it beforehand because only few households prepare it and consumers are more in number comparatively.*Haria* is easily available and inexpensive beverage of west Bengal which cost about is about twenty rupees per liter.The inexpensiveness of *Haria* is because of the short process of preparation, easily availability of raw materials throughout the year and cheap labour. *Haria* has great importance in traditional occasions. It is an important and an essential item in different occasions for the people in these areas. In ceremonies like *Durga puja, kali puja,* a traditional occasion *Haria* is an essential item for the puja performed in these areas and also used in other ceremonies like birth, marriage and death ceremony.During the survey it was observed that *Dabai* has huge marketing significance, it is prepared and sold in huge amounts in different hat and bazaar of North Bengal. Due to huge market for *Dabai* and the tribes of North Bengal earn a good amount of money by selling of *Dabai.* One of the unique trends in this ethnic community is that only women are engaged in preparation and marketing or selling of *Haria* and *Dabai.*These trends are passes from womens of one generation to other generation.

3.3.4. Socio-Cultural Significance

Fermented food and beverages have strong social and ritual significance and are deep-rooted in the cultural heritage of the various ethnic groups of people of North Bengal. In these regions social activities require provision and consumption of appreciable quantities of alcoholic beverages. *Haria* and other *Desi daru* are essential to solemnize marriage ceremony,birth ceremony,death ceremony of tribal population like Adiwasi,Lakra, Saibo, Berman of West Bengal.Fermented beverages are offered to perform the "kul devi puja"/"kul devta puja", the religious practise to pray Kuldev (family God). Among the Adiwasi *Haria* is essential to serve during various cultural functions such as childrens birth ceremony they used to called these occasions as "Chathiyar" and during any kind of puja, marriage, and on any other social occasions.Among Adiwasi and Lakra community during death ceremony,*Haria* is served.The tradition of preparing and consuming the fermented beverages on any special socio-cultural occasions of tribal population of West Bengal shows the Socio-Culture significance of this kind of Alcoholic beverages to this ethnic group of population.

3.3.5. Collection of samples

Survey and collection of samples

For documentation of traditional indigenous knowledge of preparation of Haria and Dabai, different places of North Bengal have been visited. Samples of *Haria* and *Dabai* have been collected aseptically from villages and markets of the Siliguri and Jalpaiguri of North Bengal. All samples were collected aseptically in sterile bottles, Falcon tubes and poly-bags, which were placed in an ice-box sample container, and safely transported to the laboratory for analysis.(Thapa *et al.*, 2004).A total of fifteen samples of *Haria* and ten samples of *Dabai* were collected from different places and local markets of North Bengal. Six samples of *Haria* were obtained from Matigara market of Siliguri. Three samples of *Dabai* and three samples of *Haria* were collected from different places of Malbazzar of North Bengal. Six samples of *Haria* and *Dabai*

were obtained from Chandmuni, Champasari and Milanmore of Siliguri. The samples were collected in the month of September 2011-March 2012.

3.3. 6. Microbiological analysis

3.3.7. Isolation of microorganism

Ten grams of *Haria* and *Dabai* samples were homogenised properly with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution (10^{-1} to 10^{-8}) of the product in same diluent was made,Lactic acid bacteria (LAB) were isolated on MRS(De Man Roggosa and sharpe agar) (M641, HiMedia, Mumbai) this is supplemented with 1% $CaCO_3$, and is incubated in Anaerobic Gas-Pack system (LE002, HiMedia, Mumbai) under anaerobic condition at 30° C, for 48-72 hour. Aerobic mesophilic counts of the *Haria* and *Dabai* were determined using plate count agar (M091A, HiMedia, and Mumbai) is incubated at 30° C for an incubation period of 48-72 hour. The yeasts and moulds of the product were isolated yeast-malt extract (YM) agar (M424, HiMedia, Mumbai), and on potato dextrose agar (M096, HiMedia, Mumbai) and supplemented with 12 µg/ml streptomycin sulphate, 10 IU/ml benzylpenicillin and, respectively, and is incubated aerobically at 28° C for 72 hour. The of yeasts and moulds colonies were selected randomly or all sampled if the plate contained less than 10 colonies, according to the protocol of Leisner *et al.* (1997). The Purity of these isolates was checked by streaking again on fresh agar plates of the same isolation media and then sub-culturing again on respective broths/agar, this was followed by microscopic examination and other phenotypic characteristics.Microbiological data obtained were converted into the logarithms of the number of colony forming unit (cfu) per g of the sample. Identified strains of Lactic Acid Bacteria isolated from the product were preserved in MRS broth using 15 % (v/v) glycerol at –20° C (Thapa *et al.*, 2004).

3.3.8. Enumeration of bacilli

For the test, 0.85 %(W/V) sterile physiological saline was prepared and a serial dilution (10^{-2} to 10-^{8}) in the same diluent was made.Spore-forming bacilli are isolated on nutrient agar (MM02, Himedia), after inactivation of the vegetable cells by heating at 100^{0}C for 2 mins (Tamang and Nikkuni, 1996). And then incubated at 37^{0} C for 24

hours.After the colonies were observed purity of the isolates was checked by streaking again on fresh agar nutrient plates of the isolation media and sub-culturing on corresponding broth/agar, which was followed by microscopic examinations. Microbiological data thus obtained were transformed in to logarithms of the numbers of the colony forming unit (cfu) per ml of the sample.Identified strains were preserved in 15% glycerol stock at -20^0.

3.3.9. Enumeration of Total Viable count (TVC)

For the test, 0.85 %(W/V) sterile physiological saline was prepared and a serial dilution (10^{-2} to 10^{-8}) in the same diluent was made.Total viable count (TVC) was determined in the plate count agar (M091A, Himedia).The inoculated plates were incubated at 30^0 C for 48-72 hours (Sholiya *et al.*, 2009). The colonies on plates were counted and transform in to cfu/ml of the sample.

3.3.10. Biolog system

Phenotypic identification of yeast isolates were done using Biolog Identification System (Biolog Inc.) based on the utilization of 95 substrates in 96 welled plate. The metabolism of the substrates in the wells of the Biolog YT-microplates results in reduction of tetrazonium dye producing a color change, and a specific "metabolic fingerprint" obtained for each strain. This metabolic fingerprint read by Biolog Microlog Reader and compared with the database of the Biolog Microlog database software (Biolog Inc.).

The Biolog microplate bacterial identification system (Biolog Inc., USA; Oxoid GmbH, Wesel, Germany) based on the utilisation of 95 single carbon sources was used for the identification of *Leuconostoc* species. Before inoculation of the Biolog AN microplates, strains were grown anaerobically on BUA (Biolog Universal Anaerobe) agar (Oxoid GmbH, Germany) with 5 % sheep blood at 30° C for 48 hour. The bacterial cells were swabbed from the surface of the agar and suspended in AN Inoculating fluid (Biolog Inc.) at the recommended cell density. 100 µl of the bacterial suspension was pipetted in each well of the Biolog AN microplate. The microplates were incubated in an anerobic jar with a hydrogen-free anaerobic atmosphere (Oxoid

AnaeroGen; Oxoid GmbH) at 30° C for 48 hour and then read with the Biolog Microstation Reader (Biolog Inc.).

3.4. Characterization of yeast Isolates

3.4.1. 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. Dimensions of cells were measured with a standardized ocular micrometer. Identification of yeasts was carried out following the method of Kurtzman and Fell (1998) and Kreger-van Rij (1984).

3.4.2. 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) 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 coverslips 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 coverslips were examined microscopically for the formation of pseudo-mycelium or true-mycelium.

3.4.3. 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).

3.4. 4. 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 colour, 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 colour, indicating the presence of nitrate, i.e. absence of reduction (Yarrow, 1998).

3.4.5 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).

3.4. 6. 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 containing inverted Durham tubes, were inoculated with the above yeast culture and incubated at 28° C and were shaken regularly to observe gas accumulation in the inverts (Yarrow,1998).

3.4.7. Sugar assimilation

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 28° 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 Kreger-van Rij (1984), Kurtzman and Fell (1998) and Yarrow (1998).

3.4.8. (a) Determination of Ethanol tolerance capacity of isolated yeast cultures of *Haria*

Ethanol tolerance of the isolates will be determined based on visual assessment of turbidity and viability in a tube of basal medium containing 4 g/L (NH4)2SO4; 2 g/L K2O; 0.7 g/L MgSO4·7H2O; 200 g/L glucose. One hundred and fifty milli liters of prepared sterilized basal media containing known percentages of ethanol will be inoculated with actively growing yeast cells to final concentrations of 1.6×10^7 cells/ml. Observations will be made after 48 hours incubation at 25 °C. Evidence of turbidity/sedimentation will indicate growth and consequently tolerance (Nwachukwu, 2008).

(b) Determination of amylolytic activity of the yeast

Active yeast culture will be grown on the surface of soluble starch agar (4% soluble starch, 5% yeast extract, and 1.5% agar) at four positions. After incubation for 72 hours at room temperature, the petridishes are flooded with Lugols Iodine solution (2g iodine, 2gm ammonium sulphate and 300 ml distilled water) for 1 min and diameter of clear zone and colony will be measured. The amylolytic activity will be expressed as ratio of clear zone diameter to colony diameter (Limtong *et al.*, 2002).

3.4.9. Pathogenic contaminants

Enumeration of pathogenic contaminants from the samples were done in selective media such as *Bacillus cereus* agar base (M833, HiMedia, Mumbai) for *Bacillus cereus*, Baird Parker agar base (M043, HiMedia, Mumbai) for *Staphylococcus aureus* and Violet Red Bile Glucose agar w/o lactose (M581, HiMedia, Mumbai) for enterobacteriaceae (Han *et al.*, 2001). *Salmonella-Shigella* Agar (M108, HiMedia, Mumbai) was used for the detection of *Salmonella* and *Shigella* and *Listeria* identification agar base (M1064, HiMedia, Mumbai) with *Listeria* selective supplement (FD 061, HiMedia, Mumbai) for *Listeria* in the samples following the standard method of Metaxopolous *et al.* (2001). Ten grams of *Haria* and *Dabai* were blended with 90 ml of peptone-physiological saline (0.1% neutral peptone, 0.85% NaCl) and then homogenized in a stomacher lab-blender 400 (Seward, UK) for 1 min.

Then Serial dilution of the product is series was prepared in the same diluent in duplicates for its pathogenic characterization.

Bacillus cereus

Selective enumeration was carried out on spread plates of *Bacillus cereus* agar base (M833, HiMedia, Mumbai) with appropriate additions of Polymyxin B Selective Supplement (FD003, HiMedia, Mumbai) and Egg yolk emulsion (FD045, HiMedia, Mumbai). The inoculated plates were incubated at 30° C for 24-48 hours.Characteristic turquoise to peacock blue colonies surrounded by zone of precipitate of the same colour was regarded as presumptive *Bacillus cereus*.

Staphylococcus aureus

Spread plates of Baird Parker agar base (M043, HiMedia, and Mumbai) with appropriate additions of Egg yolk tellurite emulsion (FD046, HiMedia, Mumbai) was used for selective enumeration of *Staphylococcus aureus*. After serial dilution plates were overlaid with the medium and incubated at 30° C for 24-48 hours. The black colonies surrounded by clear zone extending 2-5 mm into the opaque medium appeared were regarded as presumptive *Staphylococcus aureus*.

Enterobacteriaceae

Dilutions in tryptone soya broth (M011, HiMedia, Mumbai) were allowed to resuscitate on thinly plated tryptone soya agar (M290, HiMedia, Mumbai) plates for 1-2 hour at 27° C, followed by a thick overlay of selective Violet Red Bile Glucose agar (without lactose) (M581, Himedia, Mumbai) medium and incubated at 30° C for 20 hour. Pink colonies appeared were regarded as presumptive enterobacteriaceae.

Salmonella* and *Shigella

Salmonella-Shigella (SS) agar (M108, HiMedia, and Mumbai) was used for the detection of *Salmonella* and *Shigella* in samples following the method of Metaxopolous *et al.* (2001). After serial dilution plates were inoculated, followed by an overlay of the SS agar and incubated at 37° C for 48 hour and observed in dark background for presumptive colonies. *Salmonella* colonies appear dark-centered while colorless colonies are regarded as presumptive *Shigella*.

3.5. Characterization of Bacterial Isolates

(a) Cell morphology

Smear of a 24 hour-old bacterial culture was made in a grease free slide, air-dried (not heated-fixed), stained for 30 sec with safranin (S027, HiMedia, Mumbai), washed in water, air-dried (Harrigan, 1998) and observed under oil-immersion objective. Cell dimensions were measured with a standardized ocular micrometer.

(b) CatalaseTest

The production of gas bubbles by the isolates were observed by adding 0.5 ml of 10% hydrogen peroxide solution (Merck) to the cultures indicating the presence of catalase (Schillinger and Lücke, 1987).

3.6. Biochemical analysis (Proximate Composition)

(a) pH

Ten gm of *Haria* was mixed with the 20 ml carbon dioxide-free distilled water in a blender for 1 min for mixing it properly and the pH of the slurry was determined directly by following this protocol of (AOAC, 1990) ,using a digital pH meter (Model 361, Systronics, India) and calibrated with standard buffer solutions (Merck).

(b) Titratable acidity

Titratable acidity of *Haria* was calculated by using this method of titrating the filtrates of a well blended and mixed 10 g *Haria* in 90 ml carbon-dioxide free distilled water with 0.1 N sodium hydroxide to the end point of phenolphthalein indicator (0.1 % w/v in 95 % ethanol) (AOAC, 1990).Total Acidity (TA) % is calculated by the formula :

$$TA\ \% = \frac{N \times V1 \times \text{eq.wt.of Lactic Acid} \times 100 \times \text{dilution factor}}{V2 \times 100}$$

Where N = 0.092

Eq. wt. = 90

V2 = 10

V1 = final reading of the burrete.

Dilution factor =10

(c) Moisture content

Moisture content of sample was calculated by drying 2.5–3.0 g of well-mixed sample at 135 ± 1° C for 2 hour to constant weight (AOAC, 1990).

(d) Alcohol content: Estimation of Alcohol

Alcohol content of sample was determined by dichromate oxidation method (AOAC, 1990). The 10 ml of extract was pipetted in a 500 ml round-bottomed flask where 1 g of $CaCO_3$ and 100 ml of distilled water was added and distilled. The distillate was collected for 15 min and diluted to 100 ml with distilled water (after coming to room temperature). Diluted distillate was pipetted out into a conical flask with stopper to which 10 ml of N/5 $K_2Cr_2O_7$ and 10 ml of concentrated H_2SO_4 were added and allowed to stand for 1 h. After this, stopper was removed and 100 ml of distilled water was added, followed by addition of 8 % KI and immediately titrated with N/10 $Na_2S_2O_3$ using freshly prepared 1 % starch (HiMedia RM089) solution as the indicator. Alcohol content was calculated in percentage.

Alcohol (%) = $(V_1\text{-}V_2) \times f_2 \times 0.00115 \times 100/V_3$ 100/S (multiply by 250/E of diluted extract used).

V_1 = titration volume of N/10 $Na_2S_2O_3$ against 10 ml of N/5 $K_2Cr_2O_7$ (blank test without sample)

V_2 = titration volume of N/10 $Na_2S_2O_3$ against the distillate

f_2 = factor of N/10 $Na_2S_2O_3$

100 = total volume of the distillate

V_3 = pipetting volume of the distillate for the reaction

100 = %

S = sample size

250 = total volume of the diluted extract

E = ml of extract taken for alcohol distillation.

RESULT

4. RESULT

4.1. Traditional Method of preparation

Ethnic foods are fermented naturally, except many ethnic alcoholic beverages which are prepared by using group of microorganisms in the form of dry, cereal-based starter culture. Due to diversity within the species of lactic acid bacteria and bacilli which are used for preparation of ethnic fermented foods, these type of the ethnic foods vary in their sensory characteristicsThe ethnic population uses indigenous traditional method for the preparation of fermented foods and beverages, in the North East states the ethnic population prepare the fermented foods and alcoholic beverages, have good antimicrobial activity as well as biological functions enhancing, health promoting role (Tamang *et al.,* 2011).

4.1.1. Method of preparation of *Haria*

Haria is an ethnic fermented rice beverage, consumed as a staple food beverage by the tribal community of West Bengal. The method of preparation of *Haria* includes various steps, firstly the Arba-chawal (Glutinous rice) is washed then cocked glutinous rice is cooked for about 20 min, drained off and 5 % of powdered *dabai* the starter culture is sprinkled over cooked rice, it is mixed properly and placed in a container for 1-3 days at room temperature for the saccharification process. Then, the vessel is made airtight and fermented for 2-3 days in a summer and 7-8 days in a winter. After fermentation water is added to the fermented mass, mixed properly and is filtered with a clean cotton bag and collected in a clean vessel, now the *Haria* is ready for consumption It is consumed directly as a food beverage.~~This beverage is prepared by tribal population of West Bengal.~~

4.1.2. Traditional method of *Haria* preparation:

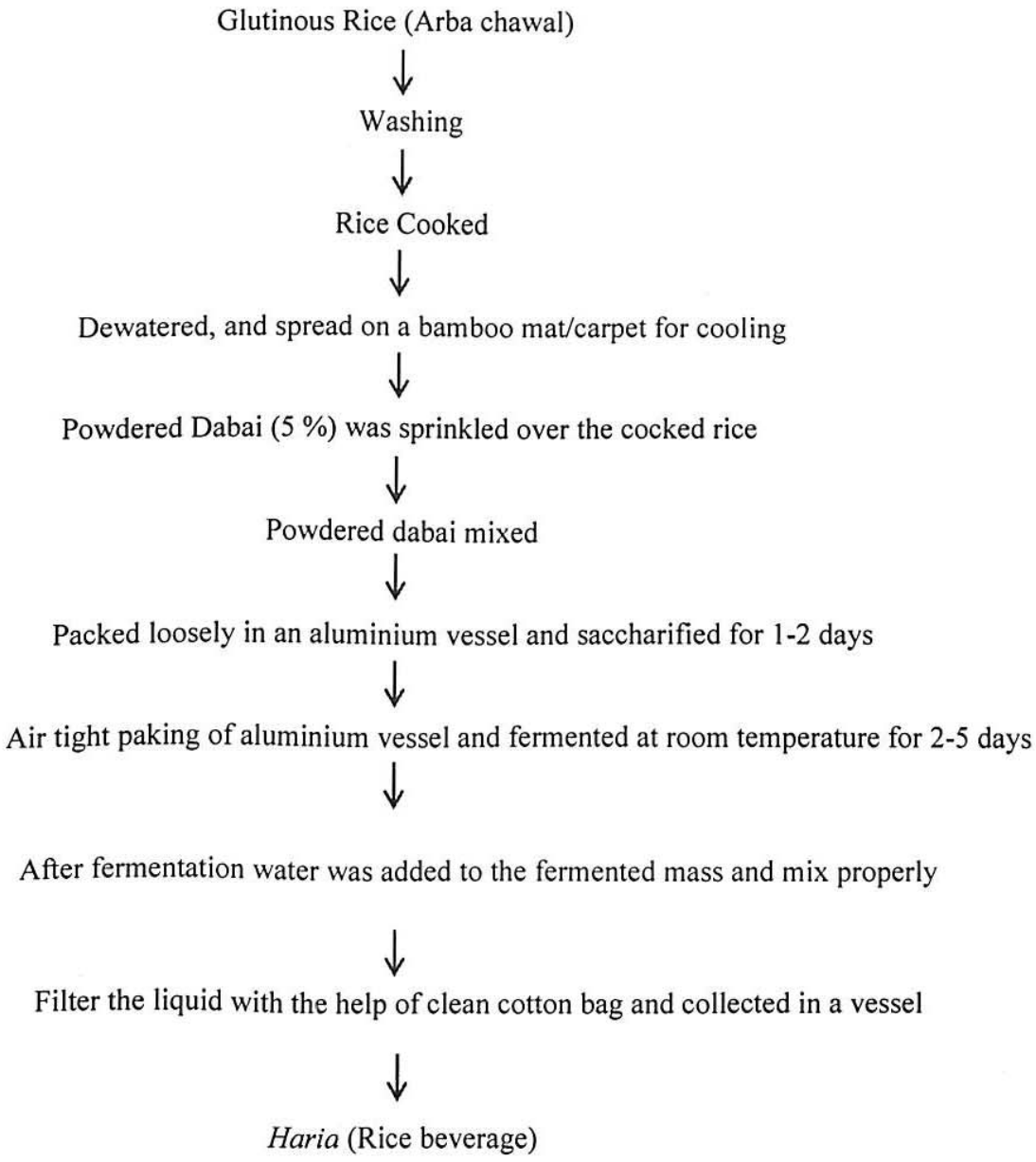


Figure 1: Flow sheet of method of *Haria* preparation in West Bengal

4.1.3. *Dabai*

Dabai is a dry, round to flattened, solid ball-like mixed dough inoculum used as starter cultures to prepare *Haria* an alcoholic beverages of West Bengal. Local varieties of rice, without soaking or soaking, and then sundry, is crushed and mixed with crushed leaves of different plants and a pinch of previously prepared powdered *Dabai*. The dough is pressed into flat and round cakes and kept over carpet and sundry for 3-5days in summer and 3-7 days in winter after sundry the starter culture *Dabai* is kept with paddy husk in a bamboo basket, and covered by clean clothes and preserved at room temperature for further use, mainly Women's are engaged in preparation and sell of *Dabai* in local markets of North Bengal.

The method for preparation of dabai is given in Figure 2.

4.1.4. Traditional method of preparation: *Dabai* is the starter culture of *Haria* is prepared by glutinous rice locally known as Arba chawal; *Dabai* is prepared by following method:

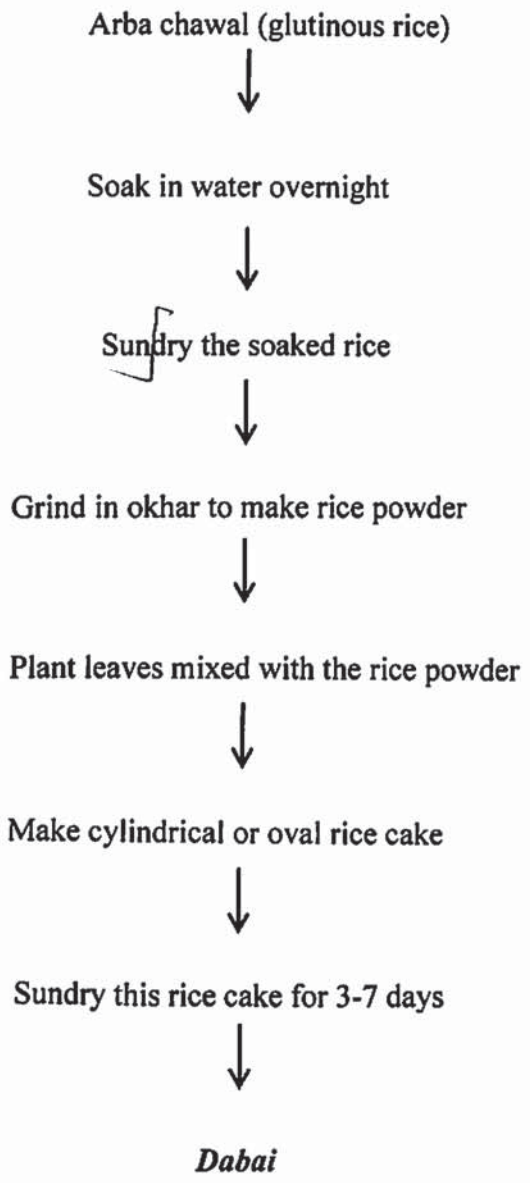


Figure 1: Flow Chart of traditional preparation of *Dabai* of West Bengal.

4.1.5. Similar fermented Alcoholic food beverages:

These are several other fermented beverages, which are presented in Table 1

Table 1. ——————— Title.

Alcoholic beverage	Substrate	Nature of product	Starter culture used	Consumers	Reference
Bhaati jaanr	Rice	Mild-alcoholic, sweet-sour, food beverage	*Marcha*	*Gorkha*	(Tamang and Thapa, 2006).
Kodo ko jaanr	Finger millet	Mild-alcoholic,sweet-sour, food beverage	*Marcha*	*Gorkha*	Tamang and Thapa, 2004).
Makai ko jaanr	Maize	Mild-alcoholic beverage	*Marcha*	*Gorkha*	(Thapa *et al.*, 2001).
Gahoon ko jaanr	Wheat	Mild-alcoholic beverage	*Marcha*	*Gorkha*	(Thapa and Tamang, 2001).
Zutho/Zhuchu	Rice	Milky white, alcoholic beverage	*Khekhrii*	*Naga*	(Teramoto *etal.*,2002)
Chhang	Finger millet/barley	Alcoholic, slightly sweet-acidic; Beverage	*Phab*	*Bhutia, Tibetan*	(Dahiya and Prabhu, 1977).

Poko	Rice	Food beverage	*Manapu*	*Gorkha*	(Shrestha *et al.*, 2002).
Atingba	Rice	Alcoholic beverage	*Hamei*	*Meitei*	Tamang *et al.*, 2007; Jeyaram *et al.*, 2008
Lao-Chao	Rice	Alcoholic beverage	Yeast cake	Chinese	(Wang and Hesseltine, 1970)
Tapé ketan	Rice	sweet/sour alcoholic	Ragi	Indionasian	(Cronk *et al.*, 1977).
Sake	Rice	sweet alcoholic	Koji	Japanese	Kodama andYoshizaw,1977)
Tapuy	Rice	sweet alcoholic	Bubod	Philipines	(Sakai and Caldo,1985)

Duizou	Red Rice	Food beverage	*Khekhrii*	*Naga*	(Tamang *et al.*, 2012).
Jou	Rice	Alcoholic beverage	*Khekhrii*	*Naga*	(Tamang *et al.*, 2012).
Madhu .	Rice	Distilled Alcoholic beverage	Yeast and Mould	Naga	(Tamang *et al.*, 2012).
Apong	Rice	sweet/sour mild alcoholic	*Ipoh*	*Monpa, Apatani,*	(Tamang *et al.*, 2012).
Pona	Rice	Alcoholic sweet-sour alcoholic	*Ipo*	*Monpa, Apatani, Nishi and Adi*	(Tamang *et al.*,2012)
Oh	Rice-millet	sweet soft alcoholic beverage	*Ipoh/Siye*	*Monpa, Apatani, Nishi, Adi*	(Tamang *et al.*,2012)

Duizou	Red Rice	Food beverage	*Khekhrii*	*Naga*	(Tamang *et al.*, 2012).
Jou	Rice	Alcoholic beverage	*Khekhrii*	*Naga*	(Tamang *et al.*, 2012).
Madhu .	Rice	Distilled Alcoholic beverage	Yeast and Mould	Naga	(Tamang *et al.*, 2012).
Apong	Rice	sweet/sour mild alcoholic	*Ipoh*	*Monpa, Apatani,*	(Tamang *et al.*, 2012).
Pona	Rice	Alcoholic sweet-sour alcoholic	*Ipo*	*Monpa, Apatani, Nishi and Adi*	(Tamang *et al.*,2012)
Oh	Rice- millet	sweet soft alcoholic beverage	*Ipoh/Siye*	*Monpa, Apatani, Nishi, Adi*	(Tamang *et al.*,2012)

4.2. Ethnic significance

Fermented foods have strong socio-cultural to socio-economic important in the whole North-East region as well as Darjeeling hills of India .This reflects their culture, food style and diversity of foods prepared and consumed by thsese ethnic population.Ethnic fermented brewing is a home-based industry mainly practiced by rural ethnic group of women of Asia and Africa using their indigenous skills and knowledge of alcohol fermentation. Similarly, the local fermented alcoholic beverage has a deep-rooted cultural history for the European as well as Mediterranean ethnic people. Alcoholic

beverages are produced by starch hydrolysis, and fermentation is done by amylolytic moulds and yeasts, followed by alcohol-producing yeasts(Hesseltine1991, Lee and Lee 2002, Nout and Aidoo 2002, Samson and Hoekstra 2002).These Alcoholic beverages represents a vast range diversity of fermented products ranging from local ethnic, traditional fermented beverages, alcoholic beverages, and distilled alcoholic beverages to wine and beer (Stewart 1987).*Haria* is one of the popular traditional rice fermented alcoholic beverage of West Bengal prepared by the tribal people such as Adiwasi, Rava, Santhal, Saibo and Uraon. Preparation of *Haria* requires specific starter culture which is called as *Dabai* by the ethnic tribal people. *Haria* is prepared and consumed throughout the year. One of the most unique features of this tribe community is that only women are engaged in the *Haria* and *Dabai* preparation and its sale.These trends are followed by the one generation to other generations in the womens of this tribal community of West Bengal.The preparation and sell of fermented *Haria* and *Dabai* of is part of culture of this ethnic group of Bengal. They used to serve this alcoholic drink in different social and cultural occasions like marriage ceremony, birthday celebration, death aniversary,different festivals like Durgapuja,Kalipuja and on other special occasions.On some special occasions they used to serve a special type of long time fermented *Haria*, this kind of long fermented *Haria* containing little bit more amount of ethanol than normal *Haria* and this *Haria* is not served to children's is only served to adults.This trends of preparation traditional fermented foods and beverages by this ethnic population of West Bengal shows their skill and knowledge and proves that they are real indigenous scientists and they are able to preserve their culture and tradition for their future generations.

4.3. Myths and Beliefs Revolving the around it

On most interesting fact I have observed in the field that they have strong belief that *Haria* can cure the diseases like cholera, diarrhoea and other diseases from old persons in the tribal areas of North Bengal. They have also strong belief that this beverage can also help in proper urination.The various plants and herves that are used in the preparation of the starter culture are believed to enrich the beverage with medicinal value. These beverages also given to the new mother to regain the strength as tonic.They have strong belief that if new born baby is feeded with one or two teaspoon of *Haria*, at the birth ceremony of new baby he or she can gain a good health.

4.4. Experimental

4.4.1 Microbiological Analysis of *Haria*

It is known that the dominant microflora of most of the mild alcoholic rice beverage and its starter culture contains yeast, moulds, LAB and Bacilli. These microorganisms are essential for development of these kinds of alcoholic beverages and therefore first we checked the presence of these microorganisms by checking their total viable count and microbial load of these microorganisms in both *Haria* and *Dabai*.

4.4.2 Microbial load of *Haria*

After the Microbiological studies it was found that the dominant microflora of *Haria* are Yeasts , Moulds, Lactic Acid Bacteria and bacilli .These microflora are responsible for the fermentation and product development. The Main microflora of *Haria* was Yeasts, Moulds, LAB and Bacilli. The load of yeasts were in the ranges between 8.60-8.65cfu/ml ,moulds 8.1-8.17cfu/ml ,LAB 8.14 -8.20cfu/ml , TVC 8.60 - 8.70 cfu/ml and Bacilli ranges between 7.0 -7.2 log cfu/g ml sample.

Table: 1 Microbial load of *Haria*.

		Log cfu/ml				
Product	**Place of collection**	**Yeast**	**Moulds**	**LAB**	**TVC**	**Bacilli**
Haria	Matigara (n=3)	8.60 ±0.6	8.1 ±0.3	8.17 ±0.7	8.60 ±0.6	7.1 ±0.3
	Shivmandir (n=3)	8.60 ±0.6	8.1 ±0.0	8.14 ±0.3	8.47 ±0.3	7.1 ±0.6
	Chandmuni (n=3)	8.56 ±0.0	8.0 ±0.3	8.17 ±0.0	8.60 ±0.6	7.0 ±0.6
	Champasari (n=3)	8.60 ±0.6	8.17 ±0.4	8.17 ±0.3	8.70 ±0.3	7.1 ±0.2
	Malbazzar (n=3)	8.65 ±0.6	8.0 ±0.3	8.20 ±0.0	8.60 ±0.6	6.9 ±0.3

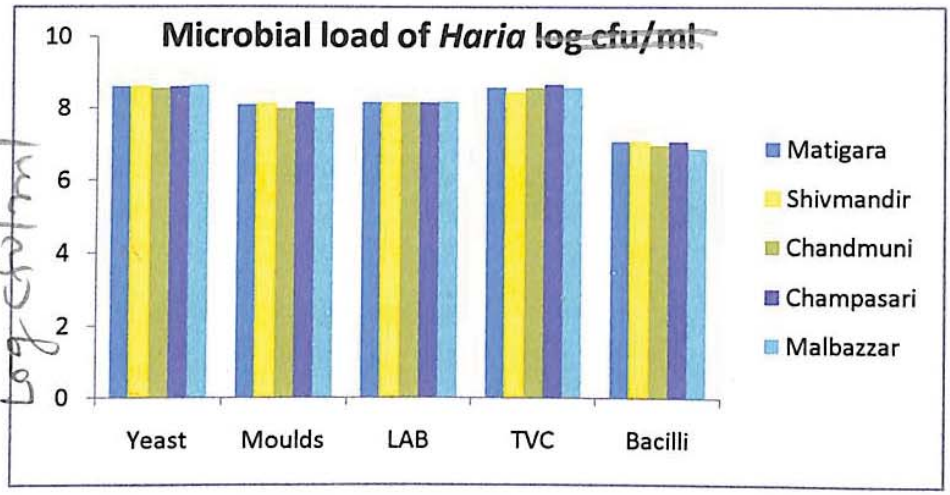


Figure 1

Graph: Microbial load of *Haria* log cfu/ml.

Table: 2. Microbial load of *Haria*

		Log cfu/ml				
Product	**Place of collection**	**Yeast**	**Moulds**	**LAB**	**TVC**	**Bacilli**
Haria	Panchanai (n=3)	8.60 ±0.6	8.1 ±0.3	8.20 ±0.3	8.60 ±0.3	7.20±0.03
	Sonapur (n=3)	8.70 ±0.3	8.3 ±0.0	8.16 ±0.6	8.70 ±0.0	7.20 ±0.06
	Islampur (n=3)	8.70±0.3	8.2 ±0.3	8.18 ±0.6	8.60 ±0.0	7.10 ±0.4
	Bidhan Nagar (n=3)	8.70 ±0.6	8.17 ±0.3	8.2 ±0.0	8.70 ±0.0	7.20±0.6
	Bagdogra (n=3)	8.70 ±0.3	8.20±0.0	8.20 ±0.7	8.60 ±0.3	7.10 ±0.5

4.4.3 Microbial load of *Dabai*

The principle microflora of *Dabai* were Yeasts, Moulds, LAB, Bacilli the load of yeasts were ranges between 8.60-8.65cfu/g, moulds 8.1-8.17cfu/g, LAB 8.14-8.20 cfu/g, TVC 8.60 -8.70cfu/gm and Bacilli ranges between 7.0 -7.2 log cfu/g of sample.

Table: 3 Microbial load of *Dabai.*

Logcfu /gm						
Product	**Place of collection**	**Yeast**	**Moulds**	**LAB**	**TVC**	**Bacilli**
Dabai (Starter culture)	Matigara (n=3)	8.60 ±0.3	8.1 ±0.3	8.17 ±0.3	8.60 ±0.3	7.1 ±0.03
	Shivmandir (n=3)	8.60 ±0.3	8.1 ±0.6	8.14 ±0.0	8.47 ±0.6	7.1 ±0.06
	Chandmuni (n=3)	8.56 ±0.3	8.0 ±0.3	8.17 ±0.3	8.60 ±0.3	7.0 ±0.4
	Champasari (n=3)	8.60 ±0.0	8.17 ±0.3	8.17 ±0.3	8.70 ±0.0	7.1 ±0.6
	Malbazzar (n=3)	8.65 ±0.3	8.0 ±0.3	8.20 ±0.6	8.60 ±0.3	7.2 ±0.5

Table: 4. Microbial population of *Dabai*.

Product	Log cfu /gm					
Dabai	**Place of collection**	**Yeast**	**Moulds**	**LAB**	**TVC**	**Bacilli**
	Panchanai (n=3)	8.60 ±0.3	8.1 ±0.3	8.1 ±0.3	8.60 ±0.3	7.1 ±0.03
	Bagdogra (n=3)	8.6 ±0.3	8.1 ±0.6	8.1 ±0.6	8.47 ±0.0	7.1 ±0.06
	Islampur (n=3)	8.7 ±0.3	8.0 ±0.3	8.1 ±0.0	8.60 ±0.0	7.0 ±0.4
	Bidhan Nagar (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

n= number of samplecollected

Data represents the mean (±SD) three sets of independent experimental results

4.4.4. The biolog identified yeast isolates of *Haria*

Strain code.	Biolog Identified strains.
HS:Y1	*Issatchenkia*
HS:Y2 N	*Candida nitratophila*
HS:Y2C	*Candida tropicalis B*
HS:Y3 IRR	*Zygosaccharomyces cidri*
HS:Y3N	*Zygosaccharomyces cidri*
HS:Y4	*Saccharomyces boulardii*
HS:Y5	*Zygosaccharomyces cidri*
HS:Y6	*Issatchenkia*
HS:Y7	*Candida musae*

4.4.5. Isolation of Microorganisms

There are different dominant microorganisms are isolated from the *Haria* and *Dabai*. There are 125 stains of yeast, 28 Lactic Acid Bacteria, 20 isolates filamentous fungi (moulds), have been isolated and preserved at -15° C in glycerolstock for further use .Nine yeast isolates of *Haria*were identified by biolog and these isolates are:(HS: Y1) *Issatchenkia, (HS: Y2 N) Candida nitratophila,* (HS: Y2C) *Candida tropicalis B, Zygosaccharomyces cidri* (HS: Y3N), *Zygosaccharomyces cidri* (HS: Y3N), *Saccharomyces boulardii* (HS:Y4), *Zygosaccharomyces cidri* (HS:Y5), *Issatchenkia*(HS:Y6), *Candida musae* (HS:Y7).

4.4.6. Colony morphology of moulds

The morphology of moulds were cottony, fuzzy, mat like with feather like structured colony present on the 72 hours old mould plates. The colonies were mainly whitish in colour.72 hours old culture showed filamented, branched like structure when observed under oil-immersion microscope.

4.4.7.Nitrate reduction test: The isolates were tested for the nitrate reduction test *Issatchenkia, Zygosaccharomyces cidri, Issatchenkia*shows negative result for the test, and rest of all strains shows positive result.

Table: 5 Nitrate reduction test of biolog confirmed strain of yeasts

Strain code	Nitrate reduction		
HS YI –C	–	–	–
HS Y2 –C	+	+	+
HS Y2 –N	+	+	+
HS Y3- IRR	–	–	–
HS Y3- N	–	–	–
HSY4	–	–	–
HSY5	+	+	+
HS Y6	–	–	–
HS Y7	+	+	+

Data represents three sets of independent experimental result

4.4.8. Growth of biolog confirmed yeast at 37^{0}C: All the isolates of which is biology confirmed *Haria* shows positive results for yeast at 37^{0}C.

Table: 6 Growth of biolog confirmed yeast isolates of *Haria* at 37^{0}C

Strain code	Growth of yeast at 37^{0}C		
HS YI –C	+	+	+
HS Y2 –C	+	+	+
HS Y2 –N	+	+	+
HS Y3- IRR	+	+	+
HS Y3- N	+	+	+
HSY4	+	+	+
HSY5	+	+	+
HS Y6	+	+	+
HS Y7	+	+	+

Data represents of three sets of independent experimental results.

4.4.9. Ascospore and Ascus morphology of yeast: These two isolates HS Y2 –N, HSY5, shows spherical ascospores and this HS Y7 strain shows elipsoidal ascospores and rest of the biolog identified strains of yeast shows globose ascospores.

Table: 7 Ascospore and Ascus of yeast isolates of *Haria*:

Strain code	Ascospore type
HS YI –C	Globose
HS Y2 –C	Globose
HS Y2 –N	Spherical
HS Y3- IRR	Globose
HS Y3- N	Globose
HSY4	Globose
HSY5	Spherical
HS Y6	Globose
HS Y7	Elipsoidal

Data represents three sets of independent experimental results.

4.4.10. Mycelium formation: All the isolates of yeast showspseudo mycelium formation except *Candida nitratophila*true mycelium formation.

Table: 8 Mycelium formations of yeast isolates of *Haria*:

Strain code	Mycelia type
HS YI –C	Pseudo
HS Y2 –C	True
HS Y2 –N	Pseudo
HS Y3- IRR	Pseudo
HS Y3- N	Pseudo
HSY4	Pseudo
HSY5	Pseudo
HS Y6	Pseudo
HS Y7	Pseudo

Data represents the means of (SD) three sets of independent experimental results.

4.4.11. Pellicle formations in yeasts:

The strains of yeast HS Y3- IRR,HS Y3- N and HSY5 shows negative result for the pellicle formation and rest of the strains shows positive result.

Table: 9 Pellicle formations in yeasts isolates of *Haria*:

Strain code	Formation of pellicle		
HS YI –C	+	+	+
HS Y2–C	+	+	+
HS Y2 –N	+	+	+
HS Y3- IRR	–	–	–
HS Y3- N	–	–	–
HSY4	+	+	+
HSY5	–	–	–
HS Y6	+	+	+
HS Y7	+	+	+

Data represents the mean (±SD) of three sets of experimental independent result

4.4.12. Budding type of biolog confirmed yeast isolates of *Haria*

Most of the biolog identified strains of yeast shows multilateral budding type, except HS Y7shows unipolar and HS Y2 –C shows bipolar budding type.

Table: 10 Budding type of biolog confirmed yeast

Strain code	Budding type
HS YI –C	Multilateral
HS Y2 –C	Bipolar
HS Y2 –N	Multilateral
HS Y3- IRR	Multilateral
HS Y3- N	Multilateral
HSY4	Multilateral
HSY5	Multilateral
HS Y6	Multilateral
HS Y7	Unipolar

Data represents the three sets of experimental results.

4.4.13. Colony morphology of the yeast isolates of *Haria*

Most of the isolates of yeast showscreamy white circular, milky white round and the cell size of the different strains are listed below.

Strain code	Origin of strains	Colony morphology	Cell size (um)
HS YI -C	*Haria*	creamy white , circular	l = 11.0 (5.4 – 21.2) b = 4.0 (3.1 – 6.8)
HS Y2 - C	*Haria*	milky white round	l = 8.4 (4.5 – 12.3) b = 4.5 (3.0 – 5.6)
HS Y2 – N	*Haria*	milky white round	l = 4.2 (2.4 – 6.2) b = 3.8 (3.0 – 5.8)
HS Y3- IRR	*Haria*	milky white round	l = 10.2 (6.4 – 15.6) b = 6.0 (3.2 – 8.3)
HS Y3- N	*Haria*	milky white round	l = 10.2 (6.4 – 15.6) b = 6.0 (3.2 – 8.3)
HSY4	*Haria*	milky white round	l = 10.2 (6.4 – 15.6) b = 6.0 (3.2 – 8.3)
HSY5	*Haria*	milky white round	l = 10.2 (6.4 – 15.6) b = 6.0 (3.2 – 8.3)
HS Y6	*Haria*	milky white round	l = 10.2 (6.4 – 15.6) b = 6.0 (3.2 – 8.3)
HS Y7	*Haria*	milky white round	l = 10.2 (6.4 – 15.6) b= 6.0(3.2-8.3)

4.4.14. Sugar fermentation

All the yeast isolates of *Haria* were ferments all the sugar, The result found is shown in the table. Except strain HS: Y5 is not fermenting the sugar raffinose.

Table: 12Sugar fermentation test of biolog identified strain of yeasts:

Strain code	Sugars fermented						
	Galactose	Maltose	Lactose	Sucrose	Dextrose	Trehalose	Raffinose
HS YI –C	+	+	+	+	+	+	+
HS Y2 –C	+	+	+	+	+	+	+
HS Y2 –N	+	+	+	+	+	+	+
HS Y3-IRR	+	+	+	+	+	+	+
HS Y3 –N	+	+	+	+	+	+	+
HS Y4	+	+	+	+	+	+	+
HS-Y5	+	+	+	+	+	+	–
HSY6	+	+	+	+	+	+	+
HS Y7	+	+	+	+	+	+	+

4.4.15. Sugar assimilation

All the yeast isolates of *Haria* showed positive results for sugar assimilation except the HSY1-C it not assimilated the Galactose and Arabinose and the HSY3-IIR it not assimilated the Arabinose sugar. The result is showed in the table: 13

Table: 13. Sugar assimilation test of biolog identified strain of yeasts

Strain code	Sugars assimilated							
	Galactose	**Maltose**	**Lactose**	**Sucrose**	**Trehalose**	**Raffinose**	**Xylose**	**Arabinose**
HS YI – C	–	+	+	+	+	+	+	–
HS Y2 – C	+	+	+	+	+	+	+	+
HS Y2 – N	+	+	+	+	+	+	+	+
HS Y3-IRR	+	+	+	+	+	+	+	–
HS Y3 – N	+	+	+	+	+	+	+	+
HS Y4	+	+	+	+	+	+	+	+
HS-Y5	+	+	+	+	+	+	+	+
HSY6	+	+	+	+	+	+	+	+
HS Y7	+	+	+	+	+	+	+	+

Data represents the three sets of experimental results.

4.4.16. Ethanol tolerance capacity of Yeast

The biolog confirmed isolated strains of yeast from *Haria* were *Zygosaccharomyces cidri, Saccharomyces boulardii, Issatchenkia, Candida musae, Candida nitrophila, Candida tropicalis B* have been checked for their ethanol tolerance capacity, all of these were showed up to 17 % of the ethanol tolerance activity.Results were showed in the table. 14.

Table: 14. Ethanol tolerance capacity of biology confirmed yeast.

Strain code	**Ethanol tolerance capacity in terms of (%) percentage**									
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**17**
HS YI –C	+	+	+	+	+	+	+	+	+	+
HS Y2 –C	+	+	+	+	+	+	+	+	+	+
HS Y2 –N	+	+	+	+	+	+	+	+	+	+
HS Y3-IRR	+	+	+	+	+	+	+	+	+	+
HS Y3 –N	+	+	+	+	+	+	+	+	+	+
HS Y4	+	+	+	+	+	+	+	+	+	+
HS-Y5	+	+	+	+	+	+	+	+	+	+
HSY6	+	+	+	+	+	+	+	+	+	+
HS Y7	+	+	+	+	+	+	+	+	+	+

Data represents the means of (SD) three sets of independent experimental results.

4.4.17. Amylolytic activity of the yeasts

The isolated strains of yeasts from *Haria* were *Zygosaccharomyces cidri, Saccharomyces boulardii, Issatchenkia, Candida musae, Candida nitrophila, Candida tropicalis B* have been checked for amylolytic activity, all the strains of yeast which were biolog confirmed showed good amylolytic activity, The other strains which were isolated from different samples of *Haria* and *Dabai* also showed good amylolytic activity

Table: 15. Amylo lytic activity of biolog confirmed yeast isolates of *Haria*:

Strain code	**Amylolytic activity**		
HS YI –C	+	+	+
HS Y2 –C	+	+	+
HS Y2 –N	+	+	+
HS Y3- IRR	+	+	+
HS Y3 –N	+	+	+
HS Y4	+	+	+
HS-Y5	+	+	+
HSY6	+	+	+
HS Y7	+	+	+

Data represents the means of three sets of independent experimental results

4.4.18. Pathogenic Contaminants of *Haria*: Table 17 shows negative results for the pathogenic contaminants for Chandmuni Matigara, Shivmandir, Champasari and Malbazzar *Haria*, that were tested during the month of December, January and March of 2011-2012. No colonies of *Bacillus cereus, Listeria Enterobacteriaceae* have been

detected from the twenty random samples collected from different part of North Bengal .It has been observed that *Enterobacteriaceae, salmonella and sheigella* were not present in the product is free of faecal contaminants which is the major cause of gastroenteritis diseases and other intestinal infections

Table: 17. Pathogenic Contaminants detection of *Haria*, during month of December of 2011.a fermented rice beverage of West Bengal.

Product name	Logcfu/ml				
	Bacillus cereus	*Enterobacteriaceae*	*Salmonella sp.*	*Shigella sp.*	*S.aureus*
Chandmuni *Haria (n=3)*	0	0	0	0	0
Shivmandir *Haria (n=5)*	0	0	0	0	0
Matigara *Haria (n=3)*	0	0	0	0	0
Champasari *Haria (n=3)*	0	0	0	0	0
Malbazzar *Haria (n=3)*	0	0	0	0	0

Data represents the mean of three sets of independent experimental results.

4.5. Biochemical analysis of *Haria*:

pH of the *Haria* Sample:

The samples were analysed it was found that the average pH of ChandmuniShivmandir,Matigara,MalbazzarChampasari*Haria* is about 3.55, 3.51, 3.52, 3.55, 3.52 respectively.

Table: 16 Average pH of the *Haria* Sample

Product	Place of collection	Average pH
Haria	Chandmuni *Haria (n=10)*	3.55±0.21
	Shivmandir*Haria (n=10)*	3.51±0.03
	Matigara *Haria (n=10)*	3.52±0.03
	Malbazzar *Haria(n=10)*	3.55±0.01
	Champasari *Haria(n=10)*	3.52±0.20

n= number of sample

Data represents the mean of three sets of independent experiments.

Titrable acidity of *Haria*

The average Titrable Acidity ofChandmuni, Matigara, Shivmandir, Champasari and Malbazzar *Haria*were 0.40%, 0.40%, 0.32%, 0.40% and 0.40% respectively.

Table: 20. Titrable acidity of *Haria* of different places of North Bengal:

Product	Place of collection	Titrable Acidity in (%)
Haria	Chandmuni	0.40%±0.01
	Shivmandir	0.32%±0.01
	Matigara	0.40%±0.01
	Champasari	0.40%±0.01
	Malbazzar	0.40%±0.01

Data represents the mean (±SD) of samples, independent results

Moisture content of *Haria:*The average moisture content of Chandmuni Matigara, Shivmandir, Champasari and Malbazzar*Haria is*80.66,80.70,81.10,and 81.20 respectively.

Table: 21. Average moisture content of *Haria* Sample:

Product	Place of collection	Average moisture content of *Haria*
Haria	Chandmuni *(n=5)*	80.66 ±3.3
	Matigara *(n=5)*	80.70±3.0

	Shivmandi *(n=5)*	81.10±3.1
	Champasari *(n=5)*	80.40±3.3
	Malbazzar *(n=5)*	81.20±3.3

Ethanol content of *Haria:* The ethanol content of Matigara, Chandmuni, Malbazzar, Shivmandir, Bagdograare *Haria* 5.72, 5.70, 5.80, 5.70 and 5.71 respectively.

Table: 22. Ethanol estimation of *Haria* by Dichromate oxidation method

Product	**Place of collection**	**% of Ethanol**
Haria	Matigara	5.72±1.90
	Chandmuni	5.70±1.41
	Malbazzar	5.80±1.65
	Shivmandir	5.70±1.0
	Bagdogra	5.71±1.50

Data represents the mean (±SD) of three independent experimental results.

4.6. Yeastcharacterization

The colony morphology of the isolated yeasts is smooth milky white in colour and irregular, milky white circular, white round in shape. When they observed under oil emrersion microscope they appeared as oval, spherical, and circular in shape. The ascospores of the biolog identified strains like *Zygosaccharomyces cidri, Issatchenkia, Saccharomyces boulardii, Candidamusae, CandidatropicalisB, Candida nitratophila*spheroidal to ellipsoidal to Globose.The biolog identified strains like *Zygosaccharomyces cidri, Issatchenkia, Saccharomyces boulardii, Candida.musae, Candida tropicalis B, Candida nitratophila* are mainly showed Psuedo myecelium only. *Candida musae* showed true myecelium.The biolog identified strains like *Zygosaccharomyces cidri, Issatchenkia, Saccharomyces boulardii, Candida musae, Candida tropicalis B, Candida nitratophila*are mainly showed multilateral budding type except *Candida musae* showed unipolar budding type and *Candida tropicalis* B showed bipolar budding type.Other strains which where manually identified showed similar colony morphology, cell morphology as well as budding type, ascospores and myecelium type.Out of around 125 isolates of yeast with the different isolates codes, HS:Y1 to HS:Y30 , CH:Y1 to CH:Y30 , BD:Y1 to BD :Y7 , SD:Y1 to SD:Y7, MD:Y1 to MD:Y30 , CD:Y1 to CD:Y20. 32 of them are *Saccharomyces,* 16 of them are *Saccharomycopsis* from the charcteristics it may have pseudo hyphae , ascospores are spheroid in shape , it may ferment the sugar ,it does not reduce nitrate and and pellicle is formed in the broth (Krutzman and Fell,1998).Likewise through such characteristics it can be said that 19 of them are *Pichia.,* 9 of the isolate are *Kluyveromyces,* 11of the isolates belongs to the genous *Shizosaccharomyces, 14* of the isolates belongs to genus of *Candia,* 20 isolates were belongs to the genus *Issatchenkia* and 2 isolates were belongs to the genus*Zygosaccharomyces* (Krutzman and Fell,1998).Out of these 125 yeast isolates around 60 isolates of yeast isolated from the *Haria* and 64 from *Dabai* samples.The yeast identified in the liquid sample of *Haria* and its starter culture *Dabai* can be thus summarized as *Saccharomyces, Kluyveromyces, Issatchenkia, Shizosaccharomyces,* , Zygosaccharomyces, *Pichia, Candia and Saccharomycopsis.* Ref Table 23

Table: 23 Phenotypic Characterization of Yeasts isolates of Fermented alcoholic beverage *Haria* and its starter culture, *Dabai* samples of West Bengal:

Identification		*Saccharomyces*	*Saccharomycopsis*	*Pichia*	*Kluyveromyces*	*Schizosaccharomyces*	*Kluyveromyces*	*Candida*	*Saccharomyces*	*Schizosaccharomyces*	*Saccharomycopsis*
Ascus and Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Growth at 37°C		+	+	+	+	+	+	+	+	+	+
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
Nitrate reduction		-	-	+	-	-	+	+	-	-	-
Sugar Assimilation	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Xylose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
Sugar	Dextrose	+	+	+	+	+	+	+	+	+	+
	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+

Strain Code	Colony morphology	Cell Morphology	Cell Size	Glucose	Raffinose	Trehalose	Lactose	Sucrose
HSY1	Creamy White round	Creamy White Spherical	L= 10 (6- 15) B=5(3-8)	+	+	+	+	+
HSY2	Creamy White round	Creamy White Oval to	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+
HSY3	Creamy White	Creamy White	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+
HSY4	Creamy White round	Creamy White Spherical	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+
HSY5	Creamy White regular	Creamy White Creamy	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+
HSY6	Creamy White regular	Creamy White Spherical	L =10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+
HSY7	Creamy White regular	Creamy White Spherical	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+
HSY8	Creamy White regular	Creamy White Oval to	L= 12 (7.5- 16) B= 7 (3- 9)	+	+	+	+	+
HSY9	Creamy White regular	Creamy White Oval to	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+
HSY10	Creamy White regular	Creamy White Spherical	=10.2(6.4– 15.6) b= 6.0(3.2 – 8.3)	+	+	+	+	+

+ means positive; - means negative; L is length; B is breadth.

Identification		*Kluyveromyces*	*Saccharomyces*	*Saccharomycopsis*	*Issatchenkia*	*Saccharomyces*	*Candida*	*Saccharomycopsis*	*Pichia*	*Saccharomyces*	*Pichia*
Ascus and Ascospore		Spheroid	Ellipsoid	Spheroid	Spheroid	Ellipsoid	Globose	Spheroid	Spheroid	Ellipsoid	Spheroid
Budding typer		Multilateral	Multilateral	multilateral	bipolar	multilateral	multilateral	multilateral	bipolar	multilateral	bipolar
Pellicle formation		+	-	+	+	-	+	+	+	-	+
Growth at 37°C		+	+	+ +	+	+	+	+	+	+	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo
Nitrate reduction		-	-	-	-	-	+	-	+	-	+
Sugar assimilation	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+

Strain code	Colony morphology	Cell morphology	Cell size	Sugar fermentation								
				Arabinose	Lactose	Sucrose	Maltose	Fructose	Trehalose	Dextrose	Trehalose	Arabinose
HSY11	Creamy White regular	Oval to cylindrical	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+	+
HSY12	Creamy White regular	Oval to cylindrical	L= = 10.(6- 15) B= B=5(3-8)	+	+	+	+	+	+	+	+	+
HSY13	Creamy White regular	White Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+	+	+
HSY14	Creamy White round	Spherical	L=10.2(6.4-15.6) B= 6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+	+
HSY15	Creamy White round	Spherical round	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+	+	+	+
HSY16	Creamy White round	Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+	+	+
HSY17	Creamy White round	Oval to cylindrical	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+	+
HSY18	Creamy White round	Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+	+
HSY19	Creamy White round	Spherical round	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+	+
HSY20	Creamy White round	Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+	+

+ means positive; - means negative; L is length; B is breadth.

Identification		*Issatchenkia*	*Saccharomyces*	*Candida*	*Issatchenkia*	*Saccharomyces*	*Candida*	*Saccharomycopsis*	*Pichia*	*Saccharomyces*	*Pichia*
Ascus and Ascospore		Spheroid	Ellipsoid	Globose	Spheroid	Ellipsoid	Globose	Spheroid	Spheroid	Ellipsoid	Spheroid
Budding typer		Bipolar	Multilateral	multilateral	bipolar	multilateral	multilateral	multilateral	bipolar	multilateral	bipolar
Pellicle formation		+	-	+	+	-	+	+	+	-	+
Growth at 37°C		+	+	+	+	+	+	+	+	+	+
Mycelium type		Pseudo	Pseudo	True	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo
Nitrate reduction		-	-	-	-	-	+	-	+	-	+
Sugar assimilation	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	+	+	+	+	+	+
Sugar	Dextrose	+	+	+	+	+	+	+	+	+	+

Strain code	Colony morphology	Cell morphology	Cell size	Arabinose	Lactose	Sucrose	Maltose	Fructose	Trehalose
HSY21	Milky white regular	Oval to cylindrical	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+
HSY22	Milky white regular	Oval to cylindrical	L= = 10.(6-15) B= B=5(3-8)	+	+	+	+	+	+
HSY23	Milky white regular	Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+
HSY24	Milky white regular	Spherical	L=10.2(6.4-15.6) B= 6.0 (3.2 – 8.3)	+	+	+	+	+	+
HSY25	Milky white regular	Spherical round	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+
HSY26	Milky white regular	Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+
HSY27	Milky white regular	Oval to cylindrical	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+
HSY28	Milky white regular	\Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+
HSY29	Creamy white round	Spherical round	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+
HSY30	Milky white regular	Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+

+ means positive; - means negative; L is length; B is breadth

Identification		*Pichia*	*Saccharomyces*	*Candida*	*Issatchenkia*	*Saccharomyces*	*Candida*	*Saccharomycopsis*	*Pichia*	*Saccharomyces*	*Pichia*
Ascus and Ascospore		Spheroid	Ellipsoid	Globose	Spheroid	Ellipsoid	Globose	Spheroid	Spheroid	Ellipsoid	Spheroid
Budding type		Bipolar	multilateral	True	bipolar	multilateral	multilateral	multilateral	bipolar	multilateral	Bipolar
Pellicle formation		+	-	+	+	-	+	+	+	-	+
Growth at 37°C		+	+	+	+	+	+	+	+	+	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo
Nitrate reduction		-	-	-	-	-	+	-	+	-	+
Sugar assimilation	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+

Strain code	Colony morphology	Cell morphology	Cell size	Sugar fermentation							
				Arabinose	Lactose	Sucrose	Maltose	Fructose	Trehalose	Dextrose	Trehalose
CH:Y1	Milky White regular	Oval to cylindrical	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+
CH:Y2	Milky White regular	Oval to cylindrical	L= = 10 (6- 15) B= B=5(3-8)	+	+	+	+	+	+	+	+
CH:Y3	Milky White regular	Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+	+
CH:Y4	Milky White round	Spherical	L=10.2(6.4-15.6) B= 6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+
CH:Y5	Milky White regular	Spherical round	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+	+	+
CH:Y6	Milky White regular	Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+	+
CH:Y7	Milky White regular	Oval to cylindrical	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+
CH:Y8	Milky White round	Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+
CH:Y9	Milky White round	Spherical round	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+
CH:Y10	Milky White round	Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+

+ means positive; - means negative; L is length; B is breadth

Identification		*Saccharomyces*	*Saccharomyces*	*Saccharomycopsis*	*I Pichia*	*Saccharomyces*	*Candida*	*Saccharomycopsis*	*ssatchenkia*	*Saccharomyces*	*Pichia*
Ascus and Ascospore		Ellipsoid	Ellipsoid	Spheroid	Spheroid	Ellipsoid	Globose	Spheroid	Spheroid	Ellipsoid	Spheroid
Budding typer		multilateral	multilateral	multilateral	bipolar	multilateral	multilateral	multilateral	bipolar	Multilateral	bipolar
Pellicle formation		–	–	+	+	-	+	+	+	-	+
Growth at 37°C		+	+	+	+	+	+	+	+	+	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo
Nitrate reduction		-	-	-	-	-	+	-	–	-	+
Sugar assimilation	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+

Strain code	Colony morphology	Cell morphology	Cell size	Sugar fermentation							
				Arabinose	Lactose	Sucrose	Maltose	Fructose	Trehalose	Dextrose	Trehalose
CH:Y11	Milky white circular	Oval to cylindrical	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+
CH:Y12	Milky white circular	Oval to cylindrical	L= = 10 (6- 15) B= B=5(3-8)	+	+	+	+	+	+	+	+
CH:Y13	Milky White	Oval to cylindrical	l=10.2(6.4– 15.6)	+	+	+	+	+	+	+	+
CH:Y14	Milky White round	White Spherical	B=6.0 (3.2– 8.3) L=10.2(6.4-15.6)	+	+	+	+	+	+	+	+
CH:Y15	Milky White regular	Spherical round	B= 6.0 (3.2 – 8.3) L=11 (5.4-21.2)	+	+	+	+	+	+	+	+
CH:Y16	Milky white circular	Oval to cylindrical	B=4(3.1-6.8) l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+	+
CH:Y17	Milky white circular	Oval to cylindrical	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+
CH:Y18	Milky White regular	Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+
CH:Y19	Milky White regular	Spherical round	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+
CH:Y20	Milky White regular	Spherical round	L=10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+

Identification		*Kluyveromyces*	*Saccharomyces*	*Issatchenkia*	*Issatchenkia*	*Saccharomyces*	*Candida*	*Saccharomycopsis*	*Pichia*	*Saccharomyces*	*Issatchenkia*
Ascus and Ascospore		Spheroid	Ellipsoid	Spheroid	Spheroid	Ellipsoid	Globose	Spheroid	Spheroid	Ellipsoid	Spheroid
Budding typer		Multilateral	Multilateral	Bipolar	bipolar	multilateral	multilateral	multilateral	bipolar	Multilateral	bipolar
Pellicle formation		+	-	+	+	-	+	+	+	-	+
Growth at 37°C		+	+	+	+	+	+	+	+	+	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo
Nitrate reduction		-	-	-	-	-	+	-	+	-	–
Sugar assimilation	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	+	+	+	+	+	+	+
Sugar ferme	Dextrose	+	+	+	+	+	+	+	+	+	+

Strain code	Colony morphology	Cell morphology	Cell size		Arabinose	Lactose	Sucrose	Maltose	Fructose	Trehalose
CH:Y21	Creamy white regular	Oval to cylindrical	L=10.2(6.4-15.6)	b=6.0 (3.2 – 8.3)	+	+	+	+	+	+
CH:Y22	Creamy white regular	Oval to cylindrical	L= = 10 (6- 15)	B= B=5(3-8)	+	+	+	+	+	+
CH:Y23	Creamy white regular	Oval to cylindrical	l=10.2(6.4– 15.6)	B=6.0 (3.2– 8.3)	+	+	+	+	+	+
CH:Y24	Creamy white regular	Spherical	L=10.2(6.4-15.6)	B= 6.0 (3.2 – 8.3)	+	+	+	+	+	+
CH:Y25	Creamy white regular	Spherical round	L=11 (5.4-21.2)	B=4(3.1-6.8)	+	+	+	+	+	+
CH:Y26	Creamy white regular	Oval to cylindrical	l=10.2(6.4– 15.6)	B=6.0 (3.2– 8.3)	+	+	+	+	+	+
CH:Y27	Milky white regular	Oval to cylindrical	L=8.4(4.5-12..3)	B= 4.5 (3-5.6)	+	+	+	+	+	+
CH:Y28	Milky white circular	Spherical round	L=10 (6- 15)	B=5(3-8)	+	+	+	+	+	+
CH:Y29	Milky white circular	Spherical round	L=8.4(4.5-12..3)	B= 4.5 (3-5.6)	+	+	+	+	+	+
CH:Y30	Milky white circular	Spherical round	L=10 (6- 15)	B=5(3-8)	+	+	+	+	+	+

Identification
Saccharomyces
Issatchenkia
Pichia
Issatchenkia
Schizosaccharomyces
Kluyveromyces
Candida

Ascus and Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose
Pellicle formation		-	+	+	+	-	+	-
Growth at 37°C		+	+	+	+	+	+	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo
Budding type		Multilateral	Bi polar	Bi polar	Bi polar	Multilateral	Multilateral	Multilateral
Nitrate reduction		-	-	+	-	-	+	+
Sugar Assimilation	Fructose	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+
	Xylose	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+
Sugar	Dextrose	+	+	+	+	+	+	+
	Fructose	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+

Strain Code	Colony morphology	Cell Morphology	Cell Size		Glucose	Raffinose	Trehalose	Lactose	Sucrose
BD:Y 1	Creamy white round	Spherical round	L= 10 (6- 15)	B=5(3-8)	+	+	+	+	+
BD:Y 2	Creamy white round	Oval to cylindrical	l=10.2(6.4– 15.6)	B=6.0 (3.2– 8.3)	+	+	+	+	+
:BDY 3	Creamy white round	Spherical round	L=8.4(4.5-12..3)	B= 4.5 (3-5.6)	+	+	+	+	+
BD:Y 4	Creamy white round	Spherical round	L=10.2(6.4-15.6)	b=6.0 (3.2 – 8.3)	+	+	+	+	+
YD:Y 5	Creamy white regular	Oval to cylindrical	l=8.4(4.5– 12.3)	b=6.0 (3.2 – 8.3)	+	+	+	+	+
BD:Y 6	Creamy white regular	Spherical round	L =10.2(6.4-15.6)	b=6.0 (3.2 – 8.3)	+	+	+	+	+
BD:Y 7	Creamy white regular	Spherical round	L=11 (5.4-21.2)	B=4(3.1-6.8)	+	+	+	+	+

Identification	*Pichia*	*Saccharomycopsis*	*Pichia*	*Saccharomyces*	*Schizosaccharomyces*	*Kluyveromyces*	*Issatchenkia*
Ascus and Ascospore	Spheroidal	Spheroidal	Spheroidal	Ellipsoidal	Globose	Spheroidal	Spheroid
Pellicle formation	+	+	+	-	-	+	+
Growth at 37°C	+	+	+	+	+	+	+
Myceliu m type	Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo
Budding type	bipolar	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Bipolar
Nitrate reduction	+	-	+	-	-	+	-
Sugar Assimilation Fructose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+
Sugar Dextrose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+

Identification	Strain Code	Colony morphology	Cell Morphology	Cell Size		Glucose	Raffinose	Trehalose	Lactose	Sucrose	Maltose
Saccharomyces	SD:Y 1	milky white reggular	Creamy White Spherical round	L= 10 (6- 15)	B=5(3-8)	+	+	+	+	+	+
Saccharomyces	SD:Y 2	milky white reggular	Creamy White Oval to cylindrical	l=10.2(6.4– 15.6)	B=6.0 (3.2– 8.3)	+	+	+	+	+	+
Pichia	:SD: Y3	Creamy white round	Creamy White Spherical round	L=8.4(4.5-12..3)	B= 4.5 (3-5.6)	+	+	+	+	+	+
Issatchenkia	SD:Y 4	Creamy white round	Creamy White Spherical round	L=10.2(6.4-15.6)	b=6.0 (3.2 – 8.3)	+	+	+	+	+	+
Schizosaccharomyces	SD:Y. 5	Creamy white round	Creamy White Oval to cylindrical	l=8.4(4.5– 12.3)	b=6.0 (3.2 – 8.3)	+	+	+	+	+	+
Kluyveromyces	SD:Y 6	Creamy white round	Creamy White Spherical round	L =10.2(6.4-15.6)	b=6.0 (3.2 – 8.3)	+	+	+	+	+	+
Candida	SD:Y 7	Creamy white round	Creamy White Spherical round	L=11 (5.4-21.2)	B=4(3.1-6.8)	+	+	+	+	+	+
Saccharomyces											
Schizosaccharomyces											
Saccharomycopsis											

Cell Size	Sugar Fermentation							Sugar Assimilation							Nitrate reduction	Budding type	Mycelium type	Growth at 37°C	Pellicle formation	Ascus and Ascospore
	Glucose	Raffinose	Trehalose	Lactose	Sucrose	Maltose	Fructose	Dextrose	Arabinose	Xylose	Lactose	Glucose	Sucrose	Maltose	Fructose						
L= 10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	Multilateral	Pseudo	+	+	Ellipsoidal
l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	Multilateral	Pseudo	+	+	Ellipsoidal
L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Bi polar	Pseudo	+	+	Spheroidal
L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	Bipolar	Pseudo	+	+	Spheroid
l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	Multilateral	True	+	-	Globose
L =10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Multilateral	Pseudo	+	+	Spheroidal
L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Multilateral	Pseudo	+	-	Globose
L= 12 (7.5- 16) B= 7 (3- 9)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	Multilateral	Pseudo	+	-	Ellipsoidal
l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	bipolar	Pseudo	+	-	Globose
=10.2(6.4– 15.6) b= 6.0(3.2 – 8.3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	Multilateral	Pseudo	+	+	Spheroidal

Strain Code	Colony morphology	Cell Morphology
MD:Y1	milky white	Spherical round
MD:Y2	milky white	Oval to cylindrical
MD:Y3	milky	Spherical round
MD:Y4	milky white reggular	Spherical round
:MDY5	Creamy White	Oval to cylindrical
:MDY6	Creamy White reggular	Spherical round
MD:Y7	Creamy White reggular	Spherical round
MD:Y8	milky white reggular	Oval to cylindrical
MD:Y9	milky white reggular	Oval to cylindrical
MD:Y10	milky white reggular	Spherical round

Identification	*Pichia*	*Saccharomycopsis*	*Pichia*	*Kluyveromyces*	*Schizosaccharomyces*	*Saccharomyces*	*Candida*	*Saccharomyces*	*Issatchenkia*	*Issatchenkia*
Ascus and Ascospore	Spheroidal	Spheroidal	Spheroidal	Spheroid	Globose	Ellipsoidal	Globose	Ellipsoidal	Spheroidal	Spheroidal
Pellicle formation	+	+	+	+	-	-	-	-	+	+
Growth at 37°C	+	+	+	+	+	+	+	+	+	+
Mycelium type	Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type	Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Multilateral	Multilateral	Multilateral	bipolar	Bipolar
Nitrate reduction	-	-	+	-	-	–	+	-	-	-
Sugar Assimilation										
Fructose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+	+	+	+	+

Strain Code	Colony morphology	Cell Morphology	Cell Size	Sugar Fermentation						
				Glucose	Raffinose	Trehalose	Lactose	Sucrose	Maltose	Fructose
MD:Y11	creamy white , circular	Spherical round	L= 10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+
MD:Y12	creamy white , circular	to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+
MD:Y13	milky white round	Spherical round	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+
MD:Y14	milky white round	Spherical round	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
:MDY15	milky white round	Oval to cylindrical	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
:MDY16	milky white round	Spherical round	L =10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
MD:Y17	creamy white , circular	Spherical round	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+	+
MD:Y18	creamy white , circular	Oval to cylindrical	L= 12 (7.5- 16) B= 7 (3- 9)	+	+	+	+	+	+	+
MD:Y19	creamy white , circular	Oval to cylindrical	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
MD:Y20	creamy white , circular	Spherical round	=10.2(6.4– 15.6) b= 6.0(3.2 – 8.3)	+	+	+	+	+	+	+

Identification		*Saccharomyces*	*Issatchenkia*	*Pichia*	*Zygosaccharomyces*	*Schizosaccharomyces*	*Issatchenkia*	*Candida*	*Saccharomyces*	*Schizosaccharomyces*	*Zygosaccharomyces*
Ascus and Ascospore		Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroid al	Globose	Ellipsoid al	Globose	Spheroid al
Pellicle formation		-	+	+	-	-	+	-	-	-	-
Growth at 37°C		+	+	+	+	+	+	+	+	+	+
Mycelium type		Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type		Multilateral	Bipolar	Bi polar	Multilateral	Multilateral	Bipolar	Multilateral	Multilateral	bipolar	Multilateral
Nitrate reduction		-	-	+	-	-	-	+	-	-	-
Sugar Assimilation	Fructose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+	+	+	+
	Glucose	+	+	+	+	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+	+	+	+	+
	Xylose	+	+	+	+	+	+	+	+	+	+
	Arabinose	+	+	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+	+	+

Strain Code	Colony morphology	Cell Morphology	Cell Size	Sugar Fermentation						
				Glucose	Raffinose	Trehalose	Lactose	Sucrose	Maltose	Fructose
MD:Y1	creamy white , circular	Spherical round	L= 10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+
MD:Y2	creamy white , circular	Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+
MD:Y3	milky white reggular	Spherical round	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+
MD:Y4	milky white reggular	Spherical round	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
:MDY5	milky white reggular	Oval to cylindrical	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
:MDY6	creamy white , circular	Spherical round	L =10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
MD:Y7 ,	milky white reggular	Spherical round	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+	+
MD:Y8	milky white reggular	Oval to cylindrical	L= 12 (7.5- 16) B= 7 (3 - 9)	+	+	+	+	+	+	+
MD:Y9	milky white reggular	Oval to cylindrical	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+
MD:Y10	milky white reggular	Spherical round	=10.2(6.4– 15.6) b= 6.0(3.2 – 8.3)	+	+	+	+	+	+	+

Identification	*Saccharomyces*	*Saccharomycopsis*	*Pichia*	*Kluyveromyces*	*Schizosaccharomyces*	*Issatchenkia*	*Candida*	*Saccharomyces*	*Schizosaccharomyces*	*Saccharomycopsis*
Ascus and Ascospore	Ellipsoidal	Spheroidal	Spheroidal	Spheroid	Globose	Spheroidal	Globose	Ellipsoidal	Globose	Spheroidal
Pellicle formation	-	+	+	+	-	+	-	-	-	+
Growth at 37°C	+	+	+	+	+	+	+	+	+	+
Mycelium type	Pseudo	Pseudo	Pseudo	Pseudo	True	Pseudo	Pseudo	Pseudo	Pseudo	Pseudo
Budding type	Multilateral	Multilateral	Bi polar	Multilateral	Multilateral	Bipolar	Multilateral	Multilateral	bipolar	Multilateral
Nitrate reduction	-	-	+	-	-	-	+	-	-	-
Sugar Assimilation										
Fructose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+

Cell Morphology	Cell Size	Sugar Fermentation							
		Glucose	Raffinose	Trehalose	Lactose	Sucrose	Maltose	Fructose	Dextrose
Spherical round	L= 10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+
Oval to cylindrical	l=10.2(6.4– 15.6) B=6.0 (3.2– 8.3)	+	+	+	+	+	+	+	+
Spherical round	L=8.4(4.5-12..3) B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+
Spherical round	L=10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+
Oval to cylindrical	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+
Spherical round	L =10.2(6.4-15.6) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+
Spherical round	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+	+	+
Oval to cylindrical	L= 12 (7.5- 16) B= 7 (3- 9)	+	+	+	+	+	+	+	+
Oval to cylindrical	l=8.4(4.5– 12.3) b=6.0 (3.2 – 8.3)	+	+	+	+	+	+	+	+
Spherical round	l=10.2(6.4– 15.6) b= 6.0(3.2 – 8.3)	+	+	+	+	+	+	+	+

Strain Code	Colony morphology
MD:Y11	creamy white , circular
MD:Y12	milky white reggular
MD:Y13	milky white reggular
MD:Y14	creamy white ,
:MDY15	creamy white , circular
:MDY16	milky white reggular
MD:Y17	milky white reggular
MD:Y18	creamy white , circular
MD:Y19	creamy white , circular
MD:Y20	creamy white , circular

Identification	Ascus and Ascospore	Pellicle formation	Growth at 37°C	Mycelium type	Budding type	Nitrate reduction	Sugar Assimilation			
							Glucose	Sucrose	Maltose	Fructose
Issatchenkia	Spheroidal	+	+	Pseudo	Bipolar	-	+	+	+	+
Saccharomycopsis	Spheroidal	+	+	Pseudo	Multilateral	-	+	+	+	+
Pichia	Spheroidal	+	+	Pseudo	Bi polar	+	+	+	+	+
Issatchenkia	Spheroid	+	+	Pseudo	Bipolar	-	+	+	+	+
Schizosaccharomyces	Globose	-	+	True	Multilateral	-	+	+	+	+
Saccharomyces	Ellipsoidal	-	+	Pseudo	Multilateral	-	+	+	+	+
Candida	Globose	-	+	Pseudo	Multilateral	+	+	+	+	+
Saccharomyces	Ellipsoidal	-	+	Pseudo	Multilateral	-	+	+	+	+
Schizosaccharomyces	Globose	-	+	Pseudo	Bipolar	-	+	+	+	+
Saccharomycopsis	Spheroidal	+	+	Pseudo	Multilateral	-	+	+	+	+

Cell Morphology	Cell Size	Sugar Fermentation											
		Gluco-se	Raffino-se	Trehalo-se	Lactose	Sucrose	Maltose	Fructo-se	Dextro-se	Arabin-ose	Xylose	Lactose
Spherical round	L= 10 (6- 15) B=5(3-8)	+	+	+	+	+	+	+	+	+	+	+
Oval to cylindrical	l=10.2(6.4– B=6.0 (3.2–	+	+	+	+	+	+	+	+	+	+	+
Spherical round	L=8.4(4.5- B= 4.5 (3-5.6)	+	+	+	+	+	+	+	+	+	+	+
Spherical round	L=10.2(6.4- b=6.0 (3.2 –	+	+	+	+	+	+	+	+	+	+	+
Oval to cylindrical	l=8.4(4.5– b=6.0 (3.2 –	+	+	+	+	+	+	+	+	+	+	+
Spherical round	L =10.2(6.4- b=6.0 (3.2 –	+	+	+	+	+	+	+	+	+	+	+
Spherical round	L=11 (5.4-21.2) B=4(3.1-6.8)	+	+	+	+	+	+	+	+	+	+	+
Oval to cylindrical	L= 12 (7.5- 16) B= 7 (3- 9)	+	+	+	+	+	+	+	+	+	+	+
Oval to cylindrical	l=8.4(4.5– b=6.0 (3.2 –	+	+	+	+	+	+	+	+	+	+	+
Spherical round	=10.2(6.4– b= 6.0(3.2 –	+	+	+	+	+	+	+	+	+	+	+

Strain Code	Colony morpholo-gy
MD:Y21	creamy white , circular
MD:Y22	milky white round
MD:Y23	milky white regular
MD:Y24	milky white regular
:MDY25	creamy white , circular
:MDY26	milky white regular
MD:Y27	creamy white , circular
MD:Y28	milky white reggular
MD:Y29	creamy white , circular
MD:Y30	milky white reggular r

4.7. Lactic Acid Bacteria

The colony morphology of the Lactic acid bacteria is tilted cream medium, large white convex and cell morphology were rod, cocci, tread like rod have been observed .The LAB load of *Haria* is 8.20±0.0 cfu/ml and the *Dabai* is about 8.20±0.0cfu/g .There are different LAB were isolated from the product but still has not been identified .Out of 28 strains of LAB and 19 strains were cocci-shaped cells and nine strains were non spore forming rods.Isolated Lactic Acid Bacteria were gram positive, catalase negative and facultative anaerobes.

Table: 24 Lactic AcidBacteria isolated from *Haria* of North Bengal

Strain code	Cell Morphology	Colony Morphology	Catatalase test
LAB 1	Cocci	tilted cream medium	–
LAB2	Cocci	tilted cream medium	–
LAB3	Cocci	large white convex	–
LAB4	Rod	large white convex	–
LAB5	slender rods	tilted cream medium	–
LAB6	Rod	tilted cream medium	–
LAB7	thread like rod	tilted cream medium	–
LAB8	Cocci	large white convex	–
LAB9	Cocci	tilted cream medium	–
LAB10	thread like rod	tilted cream medium	–
LAB11	Rod	tilted cream medium	–
LAB12	Rod	tilted cream medium	–
LAB13	Cocci	tilted white medium	–
LAB14	Cocci	tilted white medium	–

Strain code	Cell Morphology	Colony Morphology	Catatalase test
LAB 15	Cocci	tilted cream medium	–
LAB16	Cocci	tilted cream medium	–
LAB17	Cocci	large white convex	–
LAB 18	Rod	large white convex	–
LAB19	slender rods	tilted cream medium	–
LAB20	Rod	tilted cream medium	–
LAB21	thread like rod	tilted cream medium	–
LAB22	Cocci	large white convex	–
LAB23	Cocci	tilted cream medium	–
LAB24	thread like rod	tilted cream medium	–
LAB25	Rod	tilted cream medium	–
LAB26	Rod	tilted cream medium	–
LAB27	Cocci	tilted white medium	–
LAB28	Cocci	tilted white medium	–

5. PHOTOGRAPHS TAKEN DURING DOCUMENTATION OF *HARIA* AND *DABAI* PREPARATION METHOD:

Traditional method of *Dabai*preparation:

(Glutinous rice)

Glutinous Rice used in the *Dabai* preperation by the tribes of West Bengal

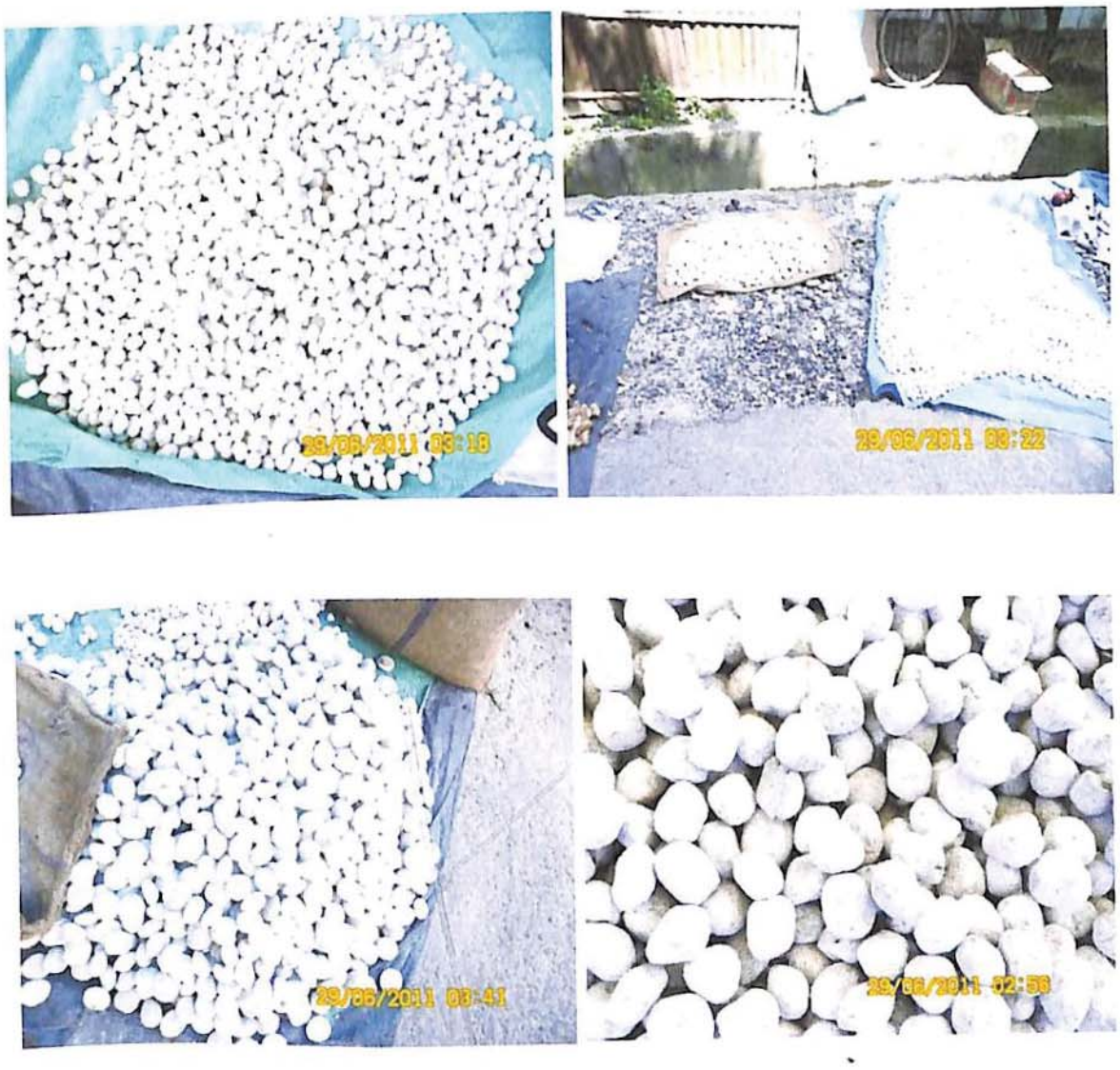
Plants leaves were mixed with the glutinous rice and grinded.

Preparation of yeast cake (*Dabai*)

(Dabai)

Photographs: Traditional Method of *Dabai* Preparation in North Bengal.

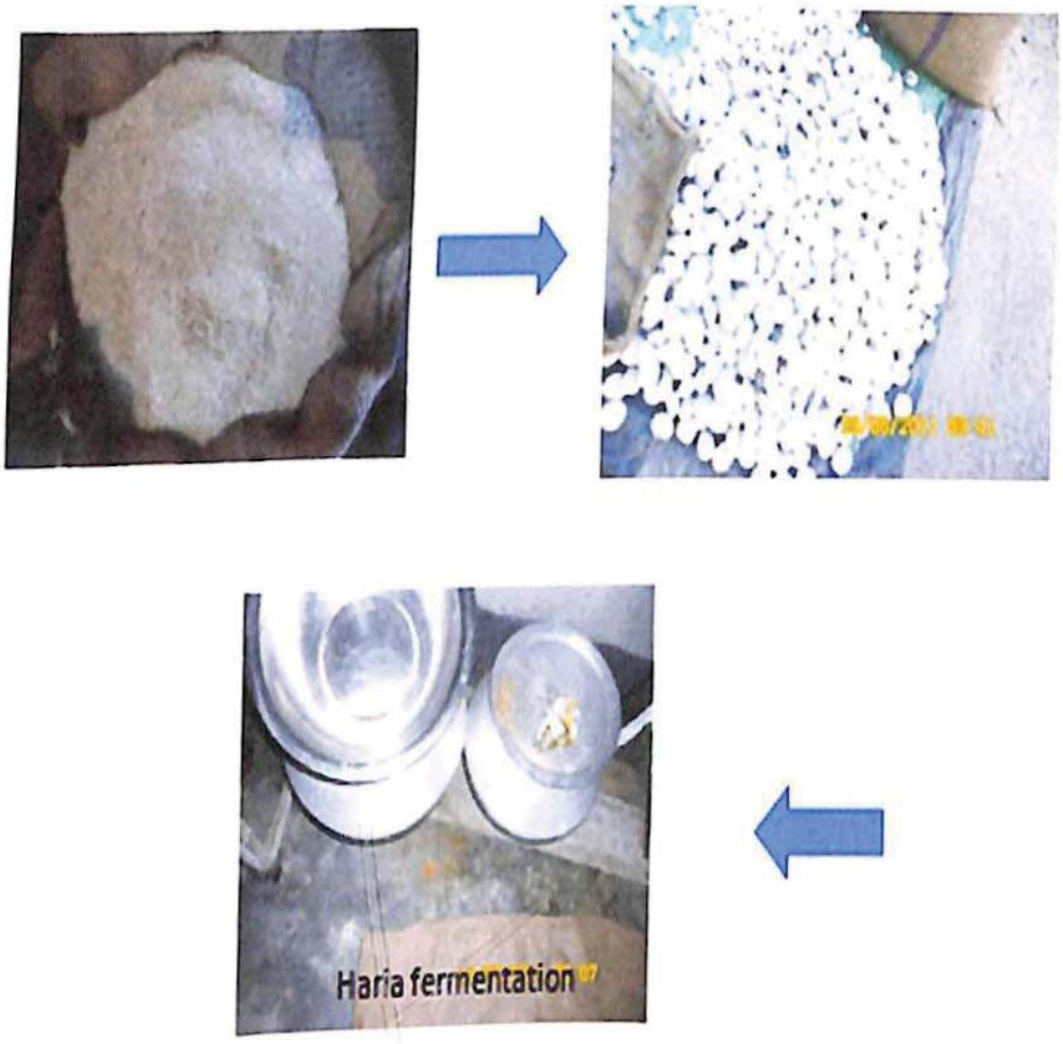
Drying proceess *Dabai* (Starter culture)



Photographs: *Dabai* was exposed to sunlight for drying

Photographs : Small sized and Midium sized *Dabai* were sold by tribal people of west bengal in Matigara Hat .

Traditional methood of fermentation of *Haria* :



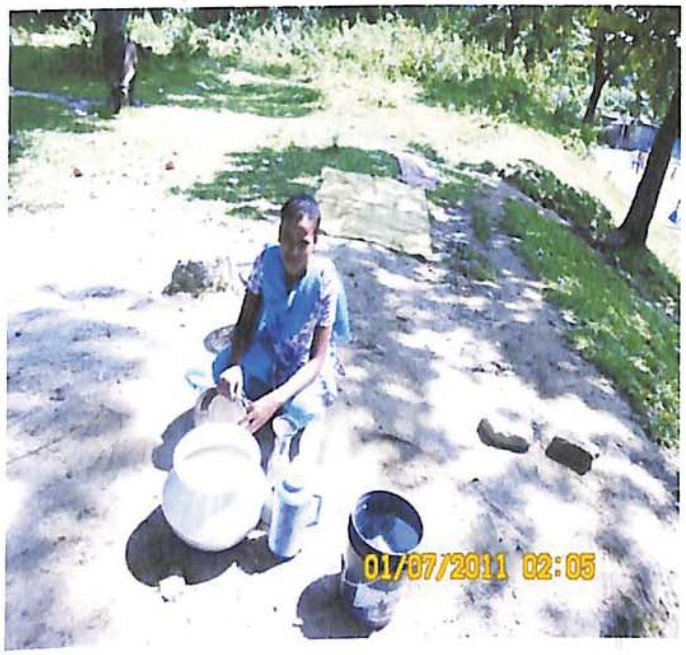
Fermentation Process

Photograph: Rice is cooked and is mixed with *Dabai*and fermented

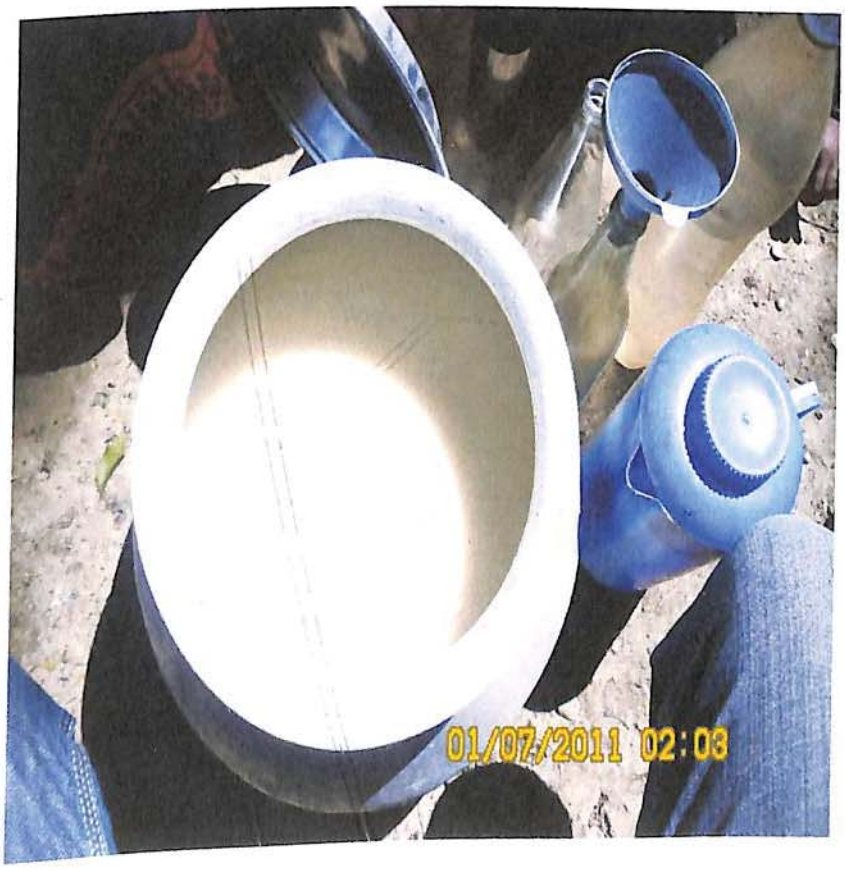
Traditional method of *Haria*preparation in the Tribal areas of North Bengal, Chandmuni.

Fermentation of glutinous rice

Fermented *Haria* ready for consumption.



Photographs: Women selling *Haria* in the Matigara region of North Bengal.



Photographs: Women selling *Haria* in Chandmuni of North Bengal .

Photographs: *Haria* is consumed by a tribal people in the Mațigara Hat of Siliguri

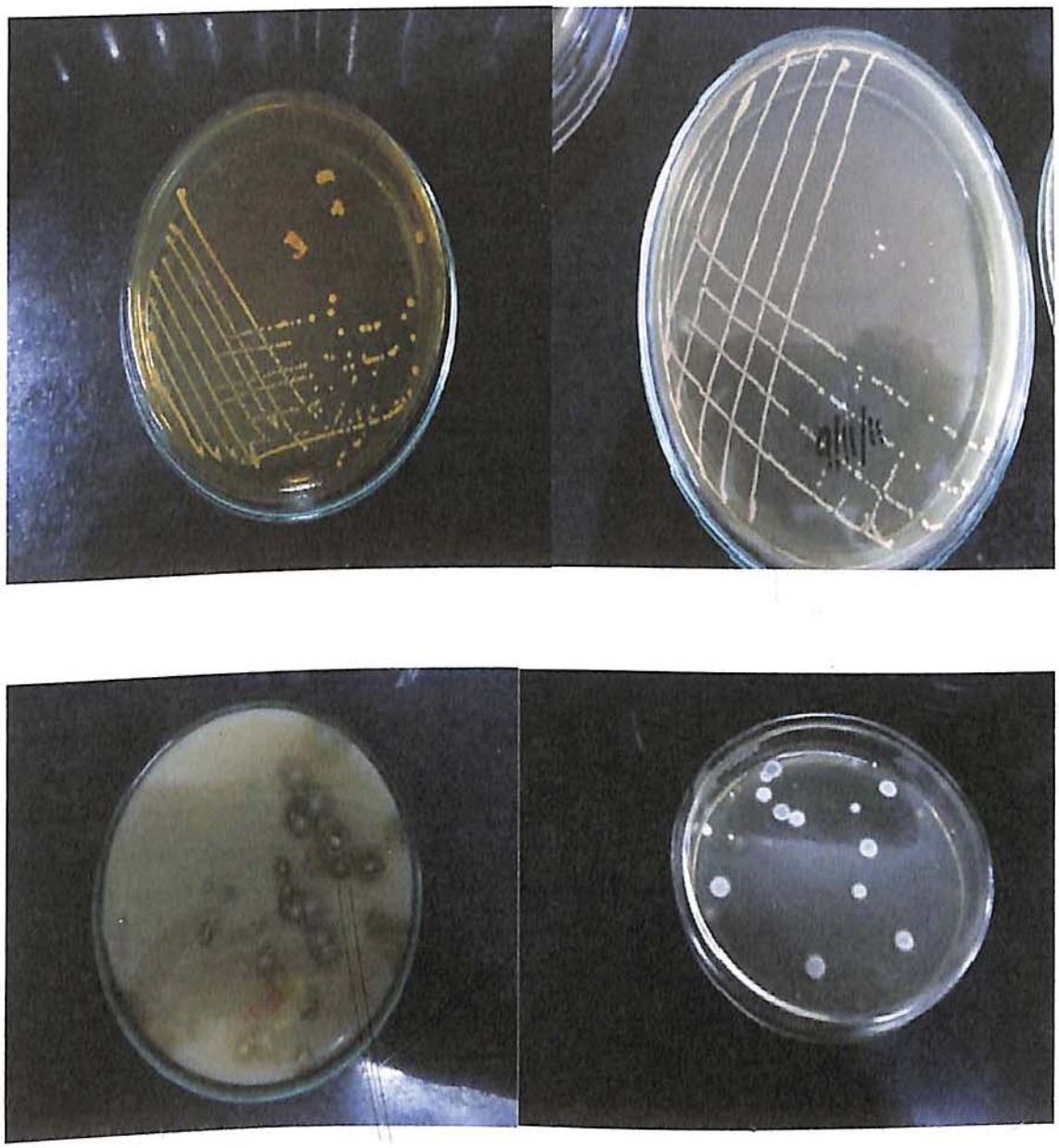
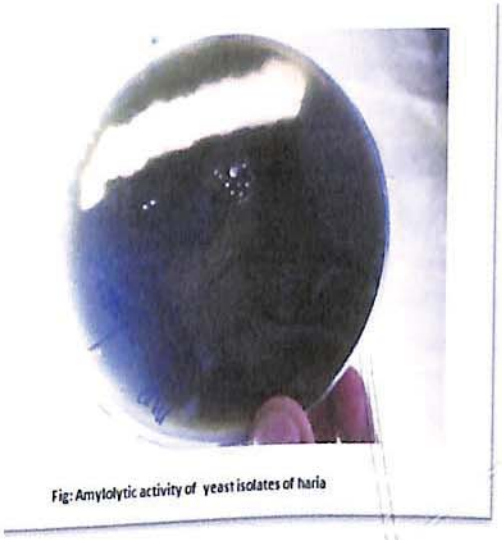


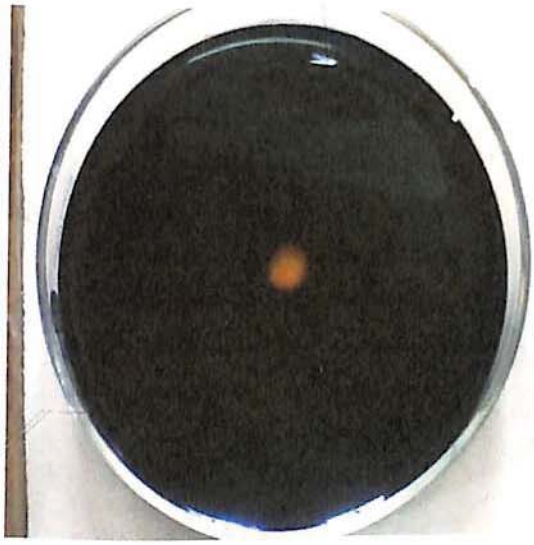
Fig: Yeast and LAB isolates of *Haria*

(a)

(b)



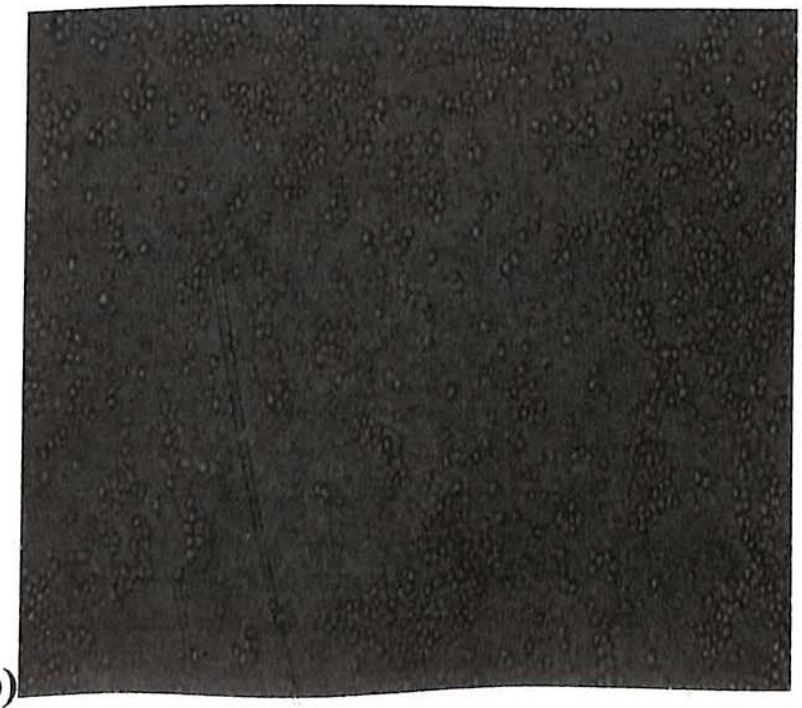
C)



(d)

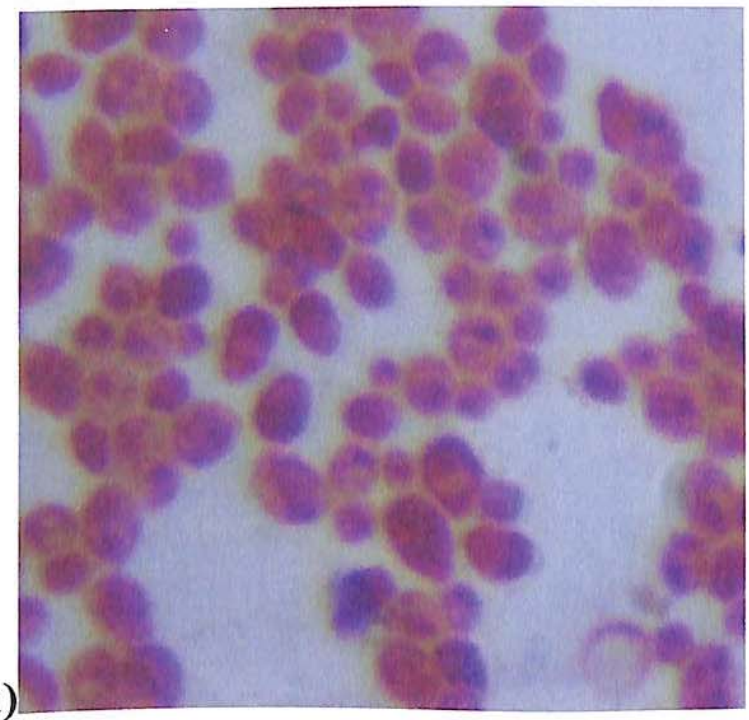
Fig: (a) Growth of yeast at 37 ^{0} C, (b) Nitrate reduction test, (c) and (d) amylolytic activity of yeast.

(a)

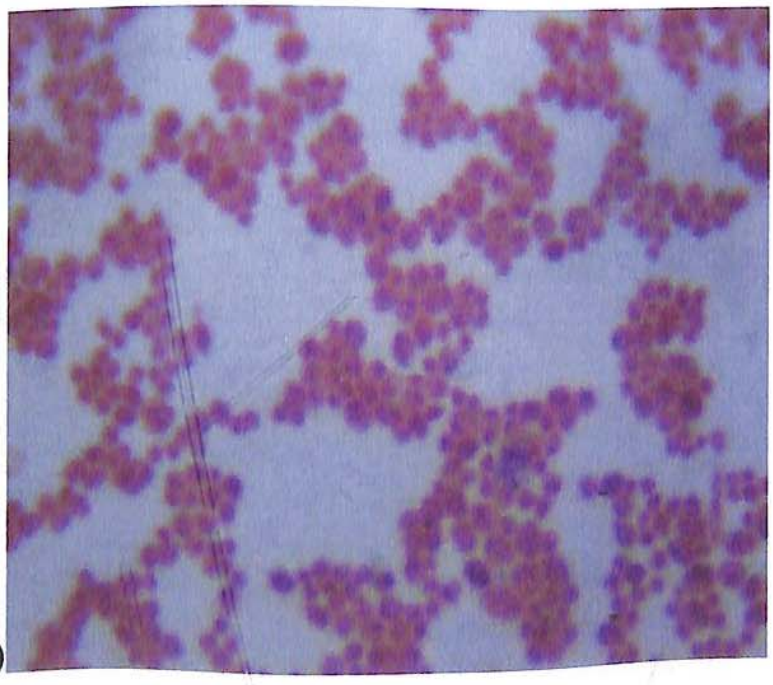


(b)

Fig: (a) and (b) Budding yeast under phase contrast microscope.

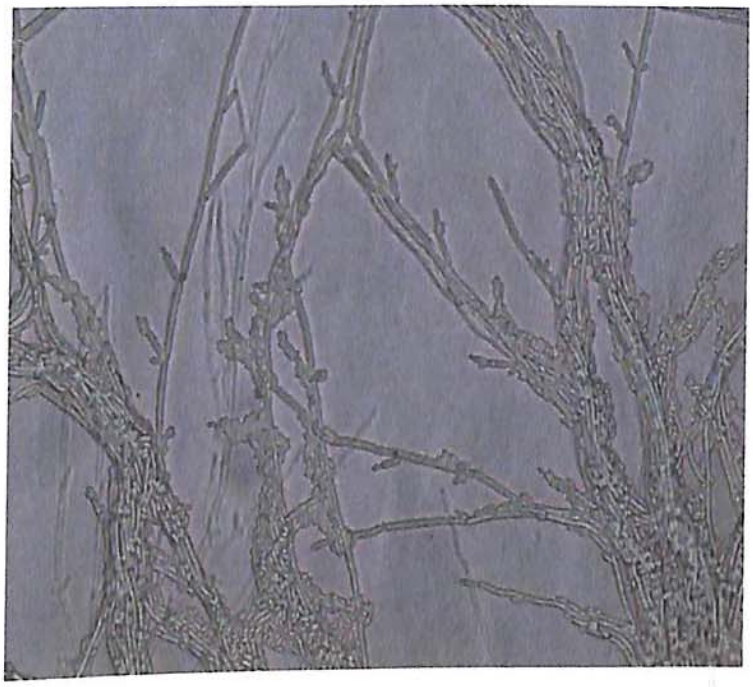


(a)



(b)

Fig: (a) and (b) Ascus& ascospore of yeasts of *Haria* and *Dabai* under phase contrast microscope



(a)

(b)

Fig: (a) and (b) Myecelium of the isolated yeast strains

DISCUSSION

Functional fermented foods and beverages provide and have several health benefitting properties which are also used in treatment and or prevention of disease. It can increase the physiological performance via some biochemical modification and/or addition of some functional ingredients (Shah, 2001).*Haria* is one of the popular traditional rice fermented alcoholic beverage prepared by the tribal people of West Bengal such as Adiwasi, Rava, Santhal, Saibo and Uraon. *Haria* is an ethnic fermented rice beverage, consumed as a staple food beverage by the tribal community of West Bengal. These kinds of fermented beverages are good source of iron, Magnesium, calcium and phosphorous (Thapa and Tamang, 2004). The indigenous knowledge was worth documenting both as means of a low cost food product and for socio-cultural reasons. The traditional method of preparation of *Haria* that was gathered during the interaction with the tribal people of West Bengal at the time of collection of samples was found to be quite similar to other documented fermented alcoholic beverages of the tribes from the North East part of India. Preparation of *Haria* requires specific starter culture which is called as *Dabai* by the local tribal people. *Haria* is prepared and consumed throughout the year. One of the most unique features of this tribe community is that only women are engaged in the *Haria* and *Dabai* preparation and its sale.All of Indian tribes prepare their indigenous traditional alcoholic beverages at home using round to flattened solid ball-like mixed dough or starter culture (Tamang *et al.*, 2007; Jeyaram *et al.*, 2008). The starter culture is also sometimes given to the cattle to provide strength in post pregnancy time. Traditional fermented foods have several beneficial and therapeutic values such as they have antioxidant, probiotic and antimicrobial properties.These fermented foods have low cholesterol level, essential amino acids, some other important health promoting compounds as well as bio-nutrients as components and thus, possibly it can act potential medicinal sources for the human health (Tamang, 2007). The Lactic acid bacteria which is most commonly found in fermented foods, provides beneficial effects like increasing the lactose digestion and preventing foods from the pathogenic contaminants (Tamang and Kailassapathy, 2010). There are several probiotic bacteria and yeast that reported in several fermented foods and beverages have several beneficial effects on the consumer health like *Lactobacilli, Bifidobacterium, Clostridium butyricum*, *Saccharomyces boulardii* are some of the important beneficial bacteria and yeasts (Prado *et al.*, 2008). Similarly, *Haria* beverage also contains yeast *Saccharomyces boulardii* may play important role in health promotion of consumers.

Functional fermented foods and beverages provide and have several health benefitting properties which are also used in treatment and or prevention of disease. It can increase the physiological performance via some biochemical modification and/or addition of some functional ingredients (Shah, 2001).*Haria* is one of the popular traditional rice fermented alcoholic beverage prepared by the tribal people of West Bengal such as Adiwasi, Rava, Santhal, Saibo and Uraon. *Haria* is an ethnic fermented rice beverage, consumed as a staple food beverage by the tribal community of West Bengal. These kinds of fermented beverages are good source of iron, Magnesium, calcium and phosphorous (Thapa and Tamang, 2004). The indigenous knowledge was worth documenting both as means of a low cost food product and for socio-cultural reasons. The traditional method of preparation of *Haria* that was gathered during the interaction with the tribal people of West Bengal at the time of collection of samples was found to be quite similar to other documented fermented alcoholic beverages of the tribes from the North East part of India. Preparation of *Haria* requires specific starter culture which is called as *Dabai* by the local tribal people. *Haria* is prepared and consumed throughout the year. One of the most unique features of this tribe community is that only women are engaged in the *Haria* and *Dabai* preparation and its sale.All of Indian tribes prepare their indigenous traditional alcoholic beverages at home using round to flattened solid ball-like mixed dough or starter culture (Tamang *et al.*, 2007; Jeyaram *et al.*, 2008). The starter culture is also sometimes given to the cattle to provide strength in post pregnancy time. Traditional fermented foods have several beneficial and therapeutic values such as they have antioxidant, probiotic and antimicrobial properties.These fermented foods have low cholesterol level, essential amino acids, some other important health promoting compounds as well as bio-nutrients as components and thus, possibly it can act potential medicinal sources for the human health (Tamang, 2007). The Lactic acid bacteria which is most commonly found in fermented foods, provides beneficial effects like increasing the lactose digestion and preventing foods from the pathogenic contaminants (Tamang and Kailassapathy, 2010). There are several probiotic bacteria and yeast that reported in several fermented foods and beverages have several beneficial effects on the consumer health like *Lactobacilli, Bifidobacterium, Clostridium butyricum, Saccharomyces boulardii* are some of the important beneficial bacteria and yeasts (Prado *et al.*, 2008). Similarly, *Haria* beverage also contains yeast *Saccharomyces boulardii* may play important role in health promotion of consumers.

The Consumption of small amounts of these mild alcoholic beverages in the form of the rice wine gives some relaxation to the hard working people of the rural and tribal areas and particularly has no side effects on their health. Apart from imparting colour, flavour and texture to the beer, the various plants used in the starter cultures are also said to have many medicinal properties. The ethnic people of NE have developed the ethnic foods to adapt to the harsh conditions and environment for centuries (Tamang *et al.,* 2010). Daily per capita consumption of ethnic fermented foods and alcoholic beverages in Sikkim is 163.8 gm representing 12.6 % of total daily diet. (Tamang *et al,* 2007). More than 250 types of ethnic fermented foods and alcoholic beverages are produced and consumed in NE as staple, curry, side dish, fried, cooked, paste, condiment, pickle, confectionery, soup, drink, masticator, alcoholic and non-alcoholic beverages (Tamang *et al.*, 2001).

Microorganisms

The microbiological analysis of *Haria* indicate that the dominant microflora of *Haria* are yeast, moulds, lactic acid bacteria and bacilli and possibly the dominant microflora responsible for fermentation of *Haria* are different types of yeast like other alcoholic beverages. In the present study we isolated more than 125 strains of yeast and out of these 120 isolates; nine isolates were selected for further study. These strains were first identified with the help of Biolog. The identification of microorganisms by Biolog uses the phenotypic characteristics of particular microorganism. The nine isolates are *Zygosaccharomycescidri, Saccharomyces boulardii, Issatchenkia, Candida musae, Candida nitrophila, Candida tropicalis B.* These strains of yeasts possibly play an important role in rice saccharification and fermentation which yield *Haria.* We also isolated moulds, lactic acid bacteria and bacilli from *Haria* and *Dabai* samples; however they are not identified and preserved for future studies. The presence of large number of yeast and moulds in *Haria* indicate that the these yeasts and moulds might be playing an important role in rice sacharification and fermentation of monosaccharides to ethanol like other alcoholic beverages whereas other microorganisms like lactic acid bacteria might be playing role in development of product flavour, aroma, texture and maintenance of acidic nature of product by producing the lactic acid. The *Lactobacillus plantarum, Pediococcus pentosaceus and Lactobacillus brevis B are* major LAB isolated from samples of starter cultures used in the states of Sikkim and Manipur for Rice wine preparation (Tamang *et al*, 2007).

Yeasts

The dominant microflora of *Haria* and *Dabai* are yeasts and moulds. The nine yeast isolates which are identified with the help of Biolog were *Zygosaccharomycescidri, Saccharomyces boulardii, Issatchenkia, Candida musae, Candida nitrophila, Candida tropicalisB.* The yeast load of the *Haria* in log cfu/ml was found to be 8.54 ± 0.0 cfu/ml to 8.65±0.3 cfu/ml. Yeast load present in the Kodo ko Jaanr is 7.1 log cfu/gm (Thapa and Tamang, 2004), in Bhaati Jaanr it is 10^7 cfu/ml (Tamang, 2010) which clearly shows that the yeast load is higher in *Haria* than *Kodo Ko Jaanr* and *Bhaati Jaanr*. After experimental analysis, the isolates of yeast were confirmed through phenotypic characterization as well as tests laid down by Kreger-Van Rig (1984), Krutzman and Fell (1998) and Yarrow (1998).The isolates were characterized on the basis of tests like nitrate reduction test, pellicle formation, characteristics of ascus and ascospores, the type of myecelium formed, sugar fermentation,sugar assimilation, growth at 37^0 C,budding type of yeast.As mentioned earlier, in the present study we isolated 125 isolates and after its characterization we found that they mainly belongs to genus *Saccharomyces, Zygosaccharomyces, Issatchenkia, Candida, Pichia, Kluyveromyces* and *Saccharomycopsis* etc. It is generally assumed that two types of Yeast are involved in *Jaanr* fermentation: amylolytic yeasts from *Marcha* (mostly *Saccharomycopsis*) degrade starch and produce glucose, andsecond group is alcohol-producing yeasts which grow rapidly on the glucose to produce ethanol (Tamang and Sarkar, 1996). So it can be assumed that the genus *Saccharomycopsis* found in the *Haria* and *Dabai* might help in the degradation or conversion of starch to glucose which will be utilized by the yeasts such as *Saccharomyces* sp. to produce ethanol. It is also reported that *Saccharomycopsis sp.* plays a significant role in the Saccharification process of rice in *Bhaati Jaanr* fermentation (Tamang and Thapa, 2006).Non –*Saccharomyces* yeast might contribute to flavour or aroma development in alcoholic beverage like, *Haria* (Rojaset *al.*, 2001). *Pichia* is an example of non-*Saccharomyces* yeasts which might help in development of sweet flavour of the beverage like *Haria*. The presence of *Kluyveromyces*have been reported in the starter culture called *Balam* which is used for the preparation of the local beverages known as *Jaan* and *Daru* prepared by the Bhotiya community of Uttaranchal (Das and Pandey, 2007). In *Bhaati Jaanr* the population of yeast is higher than the LAB and molds are not presents at the end of the fermentation product (Thapa, 2001).Like Bhaati Jaanr the yeast population of *Haria* beverage is higher than the LAB. *Zutho* is alcoholic

beverages of Nagaland *Saccharomyces cerevisiae* is the dominant microflora of this beverage (Termoto *et al.*, 2002). Out of around 125 isolates of yeasts which were labelled as HS:Y1 to HS:Y30 , CH:Y1 to CH:Y 30, BD:Y1 to BD :Y7, SD:Y1 to SD:Y7, MD:Y1 to MD:Y30 , CD:Y1 to CD:Y20; 32 of them were *Saccharomyces,* 16 of them were *Saccharomycopsis,* 19 of them were *Pichia.,* 9 of the isolate were *Kluyveromyces,* 11 of the isolates belongs to the genus *Shizosaccharomyces, 14* of the isolates belongs to genus of *Candia,* 20 isolates were belongs to the genus *Issatchenkia* and 2 isolates were belongs to the genus Zygosaccharomyces (Krutzman and Fell,1998). Out of these 125 yeast isolates around 60 isolates were from *Haria* and 64 from *Dabai* samples. Out of these *Saccharomyces*, *Saccharomycopsis Candida, Pichia* have been reported in traditional amylolytic starter culture for the preparation of alcoholic beverages like Jaanr in the hills of Darjeeling, Sikkim, Nepal, Bhutan (Tsuyoshi *et al.*, 2005).

Mycelial Fungi

Filamentous fungi in fermented foods are usually found in limited numbers. Some common genera of filamentous fungi associated with fermented foods and beverages of the world are *Actinomucor*, *Amylomyces*, *Aspergillus*, *Monascus*, *Mucor*, *Neurospora*, *Penicillium*, *Rhizopus*, and *Ustilago* (Hesseltine 1983, 1991, Samson 1993, Nout and Aidoo 2002). Filamentous fungi are mostly present in Asian fermented foods and beverages, as well as European cheese and sausages. Functional properties of fungi in fermented foods and beverages are mainly for production of enzymes such as invertase, pectinase, α-amylase, β-galactosidase, cellulase, amyloglucosidase, hemi- maltase, cellulase, and acid and alkaline proteases, lipases, and also the degradation of anti-nutritive factors, thus improving the bioavailability of minerals (Nout and Aidoo, 2002). In the present study, we found that good numbers of filamentous fungi are present in *Haria* and *Dabai;* however they were not identified and preserved for further studies.

Lactic Acid Bacteria

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 (Tamang and Kailasapathy, 2010). The LAB contains of *Bhaati Jaanr* and *Kodo ko Jaanr* are 10^4 to

10^6 cfu/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 *Bhaati Jaanr* 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 and Kailasapathy, 2010). The inability to utilize starch by *Pediococcus pentaceus* and *L.bifermentans* indicatesthat 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 (Thapa and Tamang, 2004). Similarly *Haria* 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.

Starter culture

It is not common to use starter culture for preparation of fermented foods in North East India except for alcoholic beverage production (Thapa and Tamang, 2004). The starter culture used in fermentation of *Haria* is *Dabai*, a round, oval yeast cake prepared by glutinous rice containing the microorganisms which are responsible for the fermentation of rice and product development. *Haria* prepared by using the starter culture had various advantages which results in shorter fermentation time, better hygienic condition and maintaining consistency with better quality and flavour. Modern day starter cultures are selected, either as single or multiple strains, especially for their adaption to a substrate or raw material, for example meat, cereals, milk, legumes, roots and tubers (Holzapfel *etal.*, 2003). The quality of the starter culture is dependent on the variety of plant parts used, the maintenance of proper sanitary conditions and the substrate used. It is seen that glutinous rice is preferred more than non- glutinous rice, owing to the test and alcohol content of the product. Furthermore the preparation and local marketing of this product serve as a source of income and livelihood to many of the families living in these rural areas. The starter culture or *Dabai* is prepared from several plants, in the present study some of these plants were identified, however for many other plants only local names were available. The starter

culture contains variety of yeasts however some of the important yeasts are *Saccharomyces*, *Saccharomycopsis, Candida, Pichia* which have been also reported in traditional amylolytic starter culture for the preparation of alcoholic beverages like Jaanr in the hills of Darjeeling, Sikkim, Nepal, Bhutan (Tsuyoshi *et al.,* 2005).

Pathogenic contaminants

It is very important that the foods or beverages which are consumed or sold should be free from contaminations both in terms of microbiological and chemicals also. Many diseases are transmitted due to contaminated water or samples with some pathogenic bacteria. *Haria* and Dabai are prepared and sold by people with very limited knowledge of Microbiology and therefore it is very important that both *Haria* and Dabai should be tested for the presence of pathogenic bacteria. Different samples of *Haria* and *Dabai* were tested in the lab with the selective media in order to detect the pathogenic contaminants of *Haria* and *Dabai* samples, however no pathogenic contaminants have been detected in any of the samples collected except one sample which was collected from Matigara of North Bengal have shown one colony of *Staphylococcysaureus.* These results indicate that both *Haria* and *Dabai* are free from pathogenic contaminants and possibly one colony of *Staphylococcysaureus* might be a laboratory contamination rather than the contamination of *Haria* and *Dabai*.No colonies of *Bacilluscereus*, *Listeria,Enterobacteriaceae* have been detected from the twenty random samples collected from different part of North Bengal and its surroundings which indicate that the product is of good quality and is free of pathogenic contaminants that cause fatal infections and diseases in the human beings. Absence of *Enterobacteriaceae, Salmonella* and *Shigella* ensures that the product is free of faecal contaminations which are the major cause of gastroenteritis diseases and other intestinal infections. These results indicate that that possibly *Haria* is safe from these kinds of pathogenic contamination and is acceptable for consumption for human beings. Small number of *Bacilluscereus* in foods is not considered important (Roberts *etal.*, 1996). Rapid growth of LAB is able to restrict the growth of other organisms by their physical occupation of available space and uptake of most readily assimilative nutrients (Adams and Nicolaides, 1977). Moreover, lactic acid produced by LAB reduces the pH of *Haria* to a level which can inhibit the growth of many pathogenic microorganisms (Holzapfel *et al*., 2003).

Yeast properties

Thedevelopment or production of alcoholic beverages from carbohydrate substrates requires several important characteristics in the microorganisms responsible for carrying out fermentation. Some of these properties which microorganisms should possess are good amylolytic activity, high ethanol tolerance capacity and high yield of ethanol from the substrate. In the present study we checked for some of these properties.

Amylolytic activity

The nine isolated which were confirmed by Biolog, i.e., *Zygosaccharomycescidri, Saccharomyces boulardii, Issatchenkia, Candida musae, Candida nitrophila, Candida tropicalisB* have been checked for their amylolytic activity. It was found that all the strains of yeast amylolytic activity; however the present experiment was qualitative in nature and not quantitative and therefore require other experiments to check the amylolytic activities of these isolates. The isolates having good amylolytic activity can be of industrial importance.

Ethanol tolerance capacity of Yeast

One of the important characteristics of microorganisms which are utilized for the industrial production of alcoholic beverages is that these microorganisms should have high ethanol tolerance capacity, i.e. it can survive and produce ethanol even in presence of ethanol. The nine isolates which were identified with the help of biolog been checked for their ethanol tolerance capacity and we found that these isolates can tolerate ethanol concentration up to 17 %.However further concentration are yet to be checked. This result suggest that of these isolates have good ethanol tolerance capacity and thus can be of industrial importance.

Proximate composition

In India most of the ethnic alcoholic drinks are mild alcoholic beverages having ethanol content less than 6% and all these drinks are acidic in nature. When we determined the proximate composition of *Haria,* we found that pH, titrable acidity, moisture content, alcohol content of *Haria* is 3.5, 0.32% to 0.40%, 80.66% and the ethanol content of the product is about 5.72% and thus it can be suggested that *Haria*

is a similar fermented alcoholic beverages like *Bhantti Jannr and kodo ko jannr* (Thapa and Tamang, 2004; Thapa and Tamang, 2006). The pH, titrable acidity, Moisture content and ethanol content of Kodo Ko Jaanr is 4.1, 69.7%, 0.27% and 4.8% whereas of *Bhaati Jaanr* is 3.5%, 0.24%, 83.4%, , and 5.9 % respectively (Thapa and Tamang, 2004; Thapa and Tamang, 2006). *Zutho,* a fermented rice beverage of the Nagaland, its pH and titrable acidity are 3.6% and 5.1% and the alcohol content of the product is 5%. Due to high acidic and alcoholic condition most of the bacterial and fungal species are not survived in the product *Zutho* but *Saccharomyces cerevisiae* is the dominant microflora of this beverage which plays important role in the fermentation (Termoto *et al.*, 2002).The pH of *Haria* is less than *Kodo Ko Jaanr* but, the titrable acidity, moisture content and ethanol content of *Haria* is greater than the Kodo Ko Jaanr. The PH of *Haria* is similar to *Bhaati Jaanr* however, the titrable acidity of *Haria* is greater than the *Bhaati Jaanr*, and the moisture content, ethanol content of the *Haria* is less than the Bhaati Jaanr

The proximate composition of *Haria* is very similar to many other mild alcoholic beverages and thus *Haria* can be also considered as mild alcoholic beverage. Even the microbial composition of *Haria* is similar to many mild alcoholic beverages. Some of the ethnic alcoholic beverages with higher alcohol contents are Sake, *Ruhi* and Lao-chao0. *Sake* is a rice fermented alcoholic beverage and national drink of Japan. It is a clear, pale yellow rice wine with an alcoholic content of 15 to 16% or higher, with a characteristic aroma, little acidic with slight sweetness (Murakami, 1972. Similarly, ethanol content of the *Ruhi* a fermented rice beverage ranges from 12 to 14% (v/v) with pH of 4.0 (Dahiya and Prabhu, 1977) which is laso the case for Lao-Chao of China. Malaysian Tapai contains 5% ethanol (v/v), and its pH is 3.9. Ethanol concentration of the product Tapuy is 4.93% v/v at day two of fermentation and it can reach up to 15.5% v/v at day 14 fermentation. Sanchez *et al.*, 1985 reported that eight different *bubod* starter cultures yielded ethanol contents of 12.9 to 17.3% (v/v) in tapuy. PH of the product ranged from 3.96 to 4.49. (Sakai and Caldo, 1985).The pH of *Haria* is very similar to the other alcoholic beverages of the India as well as world, but the ethanol content of Sake, Lao-Chao, Tapuy, Indian Ruhi are higher than the ethanol content of the *Haria.*

Taken these results in to account it can be suggested that the method of preparation of these fermented products, raw materials from which they are prepared and the starter culture used, *Haria* is prepared in similar manner using the same type of starter culture

for the preparation of *Bhaati Jaanr* and other rice wine of the North East India and other parts of the worlds. Even the fermentation processes to obtain these mild alcoholic beverages are very similar.

The various roles of the microorganisms in the fermentation process and further physiochemical characterisation, fermentation dynamic, its biochemistry, health promoting effects as well as other important technological properties of yeast, LAB, moulds of *Haria* and *Dabai* need to be done.

CONCLUSION

Traditional fermented foods of West Bengal is prepared at house hold level by ethnic people of the tribal and rural areas of this region by using the indigenous traditional knowledge of fermentationusing cereals as substrates for beverage preparation. The present study revealed the microbiological characteristics of fermented alcoholic rice beverage *Haria*and *Dabai*as well as their physical characteristics. The dominant micro flora ranging from filamentous yeaststomoulds and few LAB might be playing specific roles in fermentation of rice to produce *Haria*. The present study also aimed at documentation and preservation of the indigenous traditional knowledge of preparation of *Haria* and *Dabai*, its ethnic, socio-economic, socio-cultural significance.*Haria* is a low-cost mild-alcoholic, rice-based beverage consumed as a staple food-beverage in the tribal and rural belt of West Bengal. Traditional fermentations have low cost of production as it need less labour input and the (substrate) raw materials required for fermentations and product development are easily or locally available. The traditional fermented foods and beverages form a part of socio-cultural life of the tribal and rural people of the West Bengal. These fermented foods and beverages have the potential to grow in a small medium sized industry, if proper scientific and technical support is extended to the existing indigenous practices of home based fermentations. Starter culture and fermented bevearges aremostly prepared by rural women for long centuries. This art or trend of home based food fermentations technique is protected as hereditary trade and passes from mother to daughters from one generation to other generations. Microbiological studies revealed that *Sachcharomycescerevisiae, Saccharomycopsis, Saccharomyces boulardii, Zygosaccharomyces cidri, Pichia and Candida tropicalis B* are the dominant micro flora for the *Haria* fermentation and product development along with the species like *Candidamusae*, *Issatchenkia*, *Candida nitratophila* of yeast and some lactic acid bacteria (LAB). Most of the yeast isolates of the *Dabai* and *Haria* were showed amylolytic activity, and also ethanol tolerance capacity.

SUMMARY

Traditional fermented foods and alcoholic beverages are attractive alternatives to fulfil the nutritional and food requirements of large number of people. One of the famous traditional fermented alcoholic beverages of North Bengal is *Haria* which is prepared and consumed by many tribes of this region. The fermented alcoholic beverage *Haria* is unique to the tribal population like Adivasi, Lakra, Barman, Saibo, Uroa etc. of West Bengal. The traditional method of preparation *Haria* and *Dabai*, its microbiological and physical properties were studied. *Haria* is prepared by using the Arba Chawal (Glutinous rice) as substrate and *Dabai* is identified as starter culture for *Haria* fermentation. Beside other source of nutrition these fermented alcoholic beverages constitute staple food in larger part of these tribal areas of the West Bengal. People also consume *Haria* on different occasions like Marriages, birth ceremony, death ceremony and in festive occasions. Starter culture for *Haria* preparation locally known as *Dabai* is used inocula for *Haria* fermentation. Microbiological studies revealed that *Sachcharomycescerevisiae, Saccharomyces boulardii, zygosaccharomyces cidri, and Candida tropicalisB* are the dominant micro flora responsible for *Haria* fermentation and product development along with the species like *Candidamusae*, *Issatchenkia*, *Candidanitratophila* of yeast and *Pediococcus* and *Lactobacillus* (Lactic Acid Bacteria). All Yeasts isolates of the *Dabai* and *Haria* were showed amylolytic activity, and also ethanol tolerance capacity. The samples collected from various places and at different time interval were tested for presence of pathogenic contaminants like *Enterobacteriaceae*, *Bacilluscereus*, *Staphylococcus saaureus*. However, none of the samples were found to contain detected the presence of any of these pathogenic contaminants. The pH, moisture content, titrable acidity and alcohol content of the product were analysed as 3.55, 81%, 0.40% and 5.72%. The present study is preliminary in nature to find out the dominant micro flora present in *Haria* and *Dabai*, its physical properties and documentation of traditional method of its preparation. These studies can provide base line data for further investigations by researchers and scientists as well as policy makers on this kind of beverages.

BIBLIOGRAPHY

Adams, M.R. and Nicolaides, L. (1997). Review of the sensitivity different pathogens to fermentation. *Food Control* 8:227-239.

Aidoo, K. E., Nout, M.J.R. and Sarkar, P.K. (2006). Occurrence and function of yeasts in Asian indigenous fermented foods.*FEMS Yeast Research* 6:30-39.

Aderiye, B.I. and Laleye, S.A. (2003). Relevance of fermented food products in southwest Nigeria.*Plant Foods for Human Nutrition* 3:1-16.

Ananou, S., Maqueda, M., Martínez, B.M. and Valdivia, E. (2007). Biopreservation, an ecological approach to improve the safety and shelf-life of foods. In: *Communicating current research and Topics and Trends in applied Microbiology.* (Ed: A. Mendez- Vilas) Formatex, pp. 475-486.

AOAC, (1990). Official Method of Analysis, 15th Edition.Association of Official Analytical Chemists, Virginia.

Batra, L. R. (1986). Microbiology of some fermented cereals and grains legumes of India and vicinity. *In: Indigenous fermented food of non-Western origin.* (Eds: Heseltine, C. W. and Wang H. L.), Berlin, pp. 85-104.

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

Blandino, A., AlAseeri, M.E., Pandiella, S.S., Cantero, D. and Weeb, C. (2003). Cereal based fermented foods and beverages. *International Journal of Food Research* 36:527-543.

Bartholomew, J.W. (1962). Variables influencing results and the precise definition of the steps in gram staining as a mean of standardizing the results obtained. *Stain Technology* 37:139-155.

Bhatia, A.K., Singh, R.P and Atal, C.K. (1997). Chhang the fermented beverage of Himalayan Folk.*Indian Food Packer* 4:1-8.

Bhajpai, D., Sharma, A., Raghuran, N. and Bhajpai, P. (1988). Rapid Production of Ethanol in high concentration by immobilized cells of

Saccharomyces cerevisiae through soy flour supplantation. *Biotechnology Letters* 10: 217-220.

Brimer, L., Nout, M.J.R. and Tuncel, G. (1998). β-Gycosidase (amygdalase and linamarase) from *Endomyces fibuliger* (LU677): formation and crude enzyme propertirs. *Applied Microbiolog and Biotechnology* 49: 182-188.

Berger, K., U.A. Anjani, C.A. Kase, and M.J. Gaziano. (1999). Light-to-moderate alcoholConsumption and the risk of stroke among U.S. male physicians.*The New Eng Journal of Medicine* 341: 1557–1564.

Chiang, Y.W., Chye, F.Y and Ismail, A.M. (2006). Microbial Diversity and Proximate Composition of Tapai, ASabah's Fermented Beverage.*Malaysian Journal of Microbiology* 2:1-6.

Cronk, T.C., Steinkraus, K.H., Hackler, L.R and Mattick, L.R. (1977).Indonesian tape ketan fermentation.*Applied Journal of Environmental Microbiology* 33:1067-1073.

Das, C. and Pandey, A. (2007). Fermentation of traditional beverages prepared by Bhotiya community of Uttranchal Himalayas.Indian Journal of Traditional knowledge.6(1):136-140.

Dung, N.T.P., Rombouts, F.M and Nout, M.J.R. (2007). Characteristics of some Vietnamese Starch Based Fermentation starters (mem).*Lebensm-Wiss Technology* 40:130-135.

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

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

Dombek, K.M. and Ingram, L.O. (1987).Ethanol Production during Batch Fermentation with Saccharomyces cerevisiae: Changes in Glycolytic

Enzymes and Internal pH. *Journal of Applied and Environmental Microbiology* 53:1286-1291.

Das,A.J., Deka,S. C and Miiyaji,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(1) :101-107.

Deka, D. and Sarma, G.C. (2010). Traditionally used herbs in the preparation of rice-beer by the Rabha tribe of Goalpara district, Assam. *Indian Journal of Traditional Knowledge* 9(3): 459-462.

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

Dykes, G.A., Britz, T.J and VonHoly, A. (1994). Nutritional taxonomy and identification of lactic acid bacteria spoiled, vacuum packaged Vienna sausages. *Journal of Applied Bacteriology* 76:246-252.

Dahiya, D.S. and Prabhu, K.A. (1977).Indian jackfruit wine.*Symposium on Indigenous Fermented Foods*, Bangkok, Thailand.

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

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

Efiuvwevwere, B.J.O., Akhona, O. (1995). The Microbiology of Kununzaki, a cereal beverage from Nothern Nigeria, during the fermentation (production) process.*World Journal of Microbial biotechnology* 11:491-493.

Fossi, B.T., Tave, F. and Ndjouenk, R. (2004). Production and partial characterization of at hermostable amylase from ascomycetes yeast

strainisolated from starchy soils. *African Journal of Biotechnology* 4 (1):14-18.

Gordon,R.E., Haynes, W.C. and Pang, C.H-N (1973). The genus *Baccillus,*Handbook No.427.United States Development of Agriculture, WashingtonD.C.

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

Haard, N.F., Odunfa, S.A., Lee, C.H., Ramirez, Q.R., Quinones, L. and Radarte, W.C. (1999). Fermented cereals*: A Global perspective, FAO Agricultural Service Bulletin Food and Agriculture Organization Rome* 138: 63-97.

Harrigan, W.F. (1998). *Laboratory Methods in Food Microbiology,* 3rd Edition.Academic Press London.

Han, B.Z., Beumer, R.R., Rombouts, F.M and Nout, M.J.R. (2001). Microbiological safety and quality of commercial sufu –a Chinese fermented soybean food. *Food Control* 12:541-547.

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 C.P. Kurtzman. (1990). Yeasts in amylolytic food starters.*Anales deInstituto de Biología, Universidad Nacional Autónoma de México. Serie Botánica.*60: 1–7.

Holzapfel, W.H., Giesen, R., Schillinger, U., and Lücke, F.K. (2003). Starter and protective cultures. In: *FoodPreservatives*, 2nd edition, (Eds: Russell N.J. Russell and Gould G.W.), Kluwer Academic/ Plenum Publishers, New York, pp. 291-319.

Jeyaran, K., Singh, T.A., Romi, W., Devi, A.R and Singh, W.M. (2008). Traditional Fermented foods of Manipur.*Indian Journal of Traditional knoeledge* 8:115-121.

Joshi, V.K. and Pandey, A. (1999).Biotechnology. In: *Food Fermentations*. I, Educational Publishers and Distributors, New Delhi, pp: 1-24.

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

Kayode, A.P.P.,D.J.Hounhouigan,M.J.R.Nout,and A. Niehof.(2007). House hold production of sorghum beer in Benin:Technological and socio-economical aspects.*International Journal of Consumer Studies* 31: 258–264.

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

Kurtzman, C.P. and Fell, J.W. (1998). *The Yeast, A Taxonomic study*. 4thedition. Elsevier, Amsterdam.

Kodama, K. and Yoshizawa, K. (1977). Sake. In: *Economic Microbiology* (Ed: Rose, A. H.) I. Academic Press, New York, pp. 432-475.

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

Kozaki, M., J.P. Tamang, J. Kataoka, S. Yamanaka, and S. Yoshida. (2000). Cereal wine (*jaanr*) and distilled wine (*raksi*) in Sikkim. *Journal of Brewing Society of Japan* 95(2): 115–122.

Kim, C. J. (1968). Microbiological and enzymological studies on Takju brewing.*Journal of Korean Agriculture and Chemical Society* 60:69-99.

Kim, J.O. and Lee, B. H. (1970). Taxonomical studies of yeasts in Korea On yeasts isolated from Takju. *Korean Journal Microbiology* 8:77-84.

Limtong, S., Sintara S., Suwannarit, S. and Lotong, N. (2002). Yeast Diversity inThai Traditional Alcoholic Starter Kasetsart. *Journal of Natural Sciences* 36:149-158.

Lee, Y.R., Choi, Y.R., Lee, Y.S., Tae, J., Jae, P., Shim, H., Park, H.K. and Kim, Y.J. (2011). Screening Wild Yeast Strains for Alcohol fermentation from various fruits.*Journal of Mycobiology* 39(1):33-39.

Law, S. V., Abu Bakar, F., MatHashim, D. and Abdul Hamid, A. (2011). Popular fermented foods and beverages in Southeast Asia. *International Food Research Journa*l 18:475-484

Lee, C.H. and Lee, S.S. (2002). Cereal fermentation by fungi.*Applied Mycology andBiotechnology* (2): 151-170.

Lee, A.C. and Y. Fujio. (1999). Microflora of *banh men*, a fermentation starter from Vietnam. *World Journal of Microbiology and Biotechnology* 15: 51–55.

Motarjemi, Y. (2000). Impact of small scale fermentation technology on food safety in developsing countreis.*International journal of food microbiology* 75:213-229

Metaxopolous, J., Samelis, J. and Papadelli, M. (2001). Technological and microbiological evaluation of traditional process modified for industrial manufacturing of dry fermented sausages in Greece. *Italian Journal of Food Science* 13:3-18.

Murakami, H. (1972). Some problems in sake brewing. In: *Fermentation Technology.* (Ed: Terui, G.), Proc. IVth International Fermentation Symposium, Kyoto, Japan, pp. 639-643.

Mao, A. A. (1998). Etthanobotanical observation of rice beer "Zhuchu" preparation by the Mao Nagatribe from Manipur (India).*Bull bot surv India.*40 (1-4):53-57.

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

Merican, Z. and Yeoh, Q. L. (1977). Malaysian fermented foods. *Symposium on Indigenous Fermented Foods*, Bangkok, Thailand.

Mosha, T.C.E and Vicent, M.M.(2004). Nutritional value and acceptability of homemade maize/sorghum-based weaning mixtures supplemented with rojo bean flour, ground sardines and peanut paste. *International Journal of Food Sciences and Nutrition* 55(4):301-315

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose.*Journal of Biological Chemistry* 154:375-380.

Nwachukwu, I.N., Ibekwe, V.I., Nwabueze, R.N., Anyanwu, B.N., Ezeji, U., Kalu, I. and Chinakwe, E. (2008). Production of high-ethanol-yielding Saccharomyces cerevisiae of palm wine origin by protoplastfusion.*Journal of Life Science* 5:64-68.

Nout, M.J.R., Bakshi, D.and Sarkar, P.K. (1998). Microbiology safety of kinema, a fermented soyabean food. *Food Control* 9 (6):357-362.

Nout, M.J.R. and Sarkar, P.K. (1999). Lactic acid food fermentation in tropical climates.*Antonie van Leeuwenhoek*. 76(1):395-401.

Nout, M.J.R. and Motarjemi, Y. (1997). Assessment of fermentation as a household technology for improving food safety. *Food Control* 8 (5-6): 221-226.

Nout, M.J.R. and Aidoo, K.E. (2002). In: *Mycota, a Comprehensive Treatise on Fungi as Experimental Systems and Applied Research, Industrial Applications*, vol. 10, (Ed: Osiewacz, H.D.), Berlin, Germany: Springer-Verlag, pp. 23-47.

Nagai, T. and Tamang, J.P. (2010). Fermented soybeans and non-soybeans legume foods. In: *Fermented Foods and Beverages of the World.*

(Eds: Tamang, J.P. and Kailasapathy, K). CRC Press, Taylor & Francis Group, New York, pp. 191-224. **ISBN:** 9781420094954.

Ohta, K., Supanwong, K. and Hayashida, S. (1981). Environmental effects of ethanol tolerance of *Zymommonus mobilis. Journal of Bacteriology* 59:453-456.

Osho, A. (2005). Ethanol and sugar tolerance of wine yeasts isolatedfrom fermenting cashew apple juice. *African Journal of Biotechnology* 4 (7): 660-662.

Prakash, O. (1961). In: *Food and drinks in Ancient India.*Munshi Ram Monoharlal Publications, Delhi.

Prado, F.C., Parada, J.L.., Pandey, A.and Socool, C.R. (2008).Trends in non-dairy probiotic beverages. *Food Research International*4:111-123.

Park K.I., Mheen T.I., Lee K.H., Chang C.H., Lee S.R. and Kown T.W. (1977). Korean yakju and takju.*Symposium on Indigenous Fermented Foods*, Bangkok, Thailand

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.

Reiser, V. and Gasperik, J. (1995).Purification and characterization of the cell-wall-associated and extracellular alpha-glucosidases from Saccharomycopsis fibuligera.*Biochemical Journal 308*: 753-760.

Robert, T.A., Barird-Parker, A.C. and Tompkin, R.B. (1996).Microorganisms in foods,vol 5,*Microbiological Specification of Food Pathogen,*Blackie Academic and Proffessional, London.

Roy, B., Kela, C.P., Nehal, A., Faraooquee, Majila, B.S (2004).Indigenous Fermented Food and Beverages: A potential for Economic development of High Altitude Societies in Uttranchal. *Journal of Human Ecology*. 15(1):45-49.

Rojas, V., Gil, J.V., Pinaga,F., Manzanares,P. (2001). Sudies on acetate ester production by non-Saccharomyces wine yeasts. *International journal of food microbiology.*70:283-289.

Shah, N.P. (2001). Functional foods from probiotics and prebiotics.*Food Technology* .55: 46-53.

Schillinger, U. and Lucke, F.K. (1987). Identification of lactobacilli from meat and meat products.*Journal of Food Microbiology* (94): 199-208.

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): 569-565.

Samantray, G.T., Misra, P.K. and Patnaik, K.K. (1989).Mineral composition of ragi.*Indian Journal of Nutrition and Diet* 26:113-116.

Sanchez, P.C., Ramirez, T.J., and Velasco, A.C. (1985).Evaluation of the fermentation efficiency of traditional bubod for rice wine.*Philippines Journal of Agriculture* (68):483-49.

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

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

Sohliya, I., Joshi, S.R., Bhagopabaty, K.R and Kumar, R. (2009). Trungrymbai -A traditional fermented soyabean food of the ethnic tribes of Meghalaya *Indian Journal of Traditional Knowledge* (8):559-561.

Saono, S. and T. Basuki. (1978). The amylolytic, lypolytic, and proteolytic activities ofyeasts and mycelial molds from ragi and some Indonesian traditional fermentedfoods.*Annales Bogorienses* (6): 207–219.

Stewart, G.G. (1987). Alcoholic beverages. In: *Food and Beverage Mycology*, 2nd edn., (Ed: Beuchat L.R.), New York: AVI, pp. 307-354.

Samson, R.A. and Hoekstra, E.S. (2002). *Introduction to Food and Airborne Fungi*, 6th edn.Utrecht, the Netherlands: Centraalbureau voor Schimmelcultures.

Samson, R.A. (1993). The exploitation of moulds in fermented foods. In: *Exploitation of Microorganisms*, (Ed: D.G. Jone), London, U.K., Chapman and Hall, pp. 321-341.

Shrestha, H.K.N. and Rati, E.R. (2002). Microbiological profile of murcha starters and physicochemical characteristics of poko, a rice based traditional food products of Nepal. *Food Biotechnology* 16: 1-15.

Soccol, C.R., Rojan, P.J., Patel, A.K., Woiciechowski, A.L., Vandenbergh, L.P.S. and Pandey, A. (2005). Glucoamylase. In: *Enzyme Technology*. New Delhi: Asiatec Publishers Inc, pp. 221-230.

Steinkraus, K.H. (1996). *Handbook of Indigenous Fermented Foods*. 2nd Edition, Marcel Dekker, New York.

Steinkraus, K.H. (1997). Classification of fermented foods worldwide review of household fermentation techniques.*Food Control* 8(5/6): 311-317.

Steinkraus, K.H. (1977). Manufacture of primitive Thai rice wine. Symposium on Indigenous Fermented Foods. Bangkok, Thailand.

Sakai, H. and Caldo, G.A. (1983). Microbiological and chemical changes in Tapuy fermentation. In Abstracts: *The Third International Mycological Congress*, Tokyo, Japan. p 268.

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

Singh, P.K. and Singh, K.I. (2006). Traditional alcoholicbeverage, Yu of Meitei communities of Manipur.*Indian Journal of Traditional Knowledge* 5(2): 184- 190.

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): 119-125.

Tamang, J. P. (2010a). Diversity of fermented food: *Microbiology, nutrition and ethnic value*, CRC PressTalor and Francis Group, 295, ISBN: 9781420093247.

Tamang, J. P. (2010b). Diversity of fermented foods, *In: fermented foods and beverages of the world,* (eds. Tamang J.P. and Kailasapathy K), CRC Press Talor and Francis Group, 295, ISBN: 9781420094954

Tamang,J.P.,Tamang,N.,Thapa.S.,Tamang,B.,Yonzan,H.,Rai,A.K.,Chetrri,R.,Cha krabarty,J.,and Kharel, N.(2012). Microorganisms and Nutritional value of fermented foods and alcoholic beverages of North East India.*Indian Journal of Traditional Knowledge*.11 (1), 7-25.

Tamang, J.P., Sarkar, P.K., and C.W. Hesseltine. (1988). Traditional fermented foods and beverages of Darjeeling and Sikkim-A review. *Journal of Science ofFood andAgriculture* 44: 375–385.

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

Tamang, J.P. and Sarkar, P.K. (1995). Microflora of marcha: an amylolytic fermentation starter.*Microbios* 81(327): 115-122.

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, pp. 1-40.

Tamang,J.P and Nikkuni, S. (1996). Selection of starter culture for production of Kinema, a fermented fermented soyabean food of the Himalaya.*World Journal of Microbiology and Biotechnology*. 12: 629-635.

Thapa, S. and Tamang, J.P. (2004). Product characterization of kodo ko jaanr: fermented finger millet beverage of the Himalayas. *Journal of Food microbiology* 21:617-622.

Thapa, S. (2001). Microbiological and biochemical studies of indigenous fermented cereal-based beverages of the Sikkim Himalayas, *Ph.D. Thesis*; Food Microbiology Laboratory, Sikkim Government College (under North Bengal University), pp. 190.

Tamang, J.P., Thapa, S., Tamang, N. and Rai, B. (1996). Indigenous fermented food beverages of Darjeeling hills and Sikkim: Process and product characterization. *Journal of Hill Research* 9 (2):401-411.

Tamang, J.P and Thapa, S. (2006). Fermentation dynamics during production of Bhaati Jaanr, a traditional fermented Rice Beverage of the Eastern Himalayas. *Journal of Food biotechnology* 20: 251 -261.

Tamang, J.P. (2001). Food culture in the Eastern Himalayas.*Journal of Himalayan Research Cultural Foundation* 5: 107-108.

Tamang, J.P. (2007).Fermented foods for human life. In: *Microbes for human life* (Eds: Chauhan, A.K., Verma, A. and Kharakwal, H.), New Delhi, India: I.K. International Publishing, pp.73-87.

Tamang, J.P., Okumiya, K. and Kosaka, Y. (2010).Cultural Adaptation of the Himalayan Ethnic Foods with special reference to Sikkim, Arunachal Pradesh and Ladakh.*Himalayan Study Monographs of RIHN (Kyoto, Japan)* 11: 177-185.

Tamang, J.P. (2010). Benefits of traditional fermented foods.*Our World 2.0* May 5: 1-4 (www.ourworld.unu.edu).

Tongnual, P. and M.L. Fields.(1979). Fermentation and relative nutritive value of rice mealand chips.*Journal of Food Science* 44: 1784–1785

Teramoto, Y., Hano, T. and Ueda, S. (2000). Production and characteristics of an ancient form of sake made with shitogi. *Journal of the Institute of Brewing* 106(2):95-99.

Teramoto, Y., Yoshida, S. and Ueda, S. (2002). Characteristics of a rice beer (zutho) and yeast isolated from the fermented product in Nagaland, India. *International Journal of Food Microbiology* 18(9):813-816.

Thornley, M.J. (1960). The differentiation of pseudomonas from other gram negative bacteria on the basis of arginine metabolism.*Journal of applied Bacteriology* 23:37-52.

Thanh, V.N., L.T. Mai, and 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.

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* 99:135-146.

Tanimura, W., P.C. Sanchez, and M. Kozaki. (1977). The fermented food in the Philippines (part 1) tapuy (rice wine). *Journal of Agricultural Science of the Tokyo University of Agriculture* 22(1): 118–134.

Tanimura, W., P.C. Sanchez, and M. Kozaki. (1978). The fermented foods in the Philippines (Part-II).Basi (sugarcane wine).*Journal of Agricultural Society (Japan)* 22: 118–133.

Uchimura, T., Y. Niimura, N. Ohara, and M. Kozaki. (1991). Microorganisms in Luck pang used for vinegar production in Thailand.*Journal of Brewing Society of Japan* 86(1):62–67.

Vachanavinich, K., W.J. Kim, and Y.I. Park. (1994). Microbial study on krachae, Thai rice wine. In *Lactic Acid Fermentation of Non-Alcoholic Dairy Food and Beverages*, eds. Lee, C.H, J. Adler-

Nissen, and G. Bärwald, pp. 233–246. Seoul, South Korea: Ham Lim Won.

Wei, D. and S. Jong. (1983). Chinese rice pudding fermentation: Fungal flora of starter cultures and biochemical changes during fermentation. *Journal of FermentationTechnology* 61(6): 573–579.

Wang, H.L. and Hesseltine, C.W. (1970).Sufu and Lao-Chao.*Journal of Agriculture and Food Chemistry* 18:572-575.

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

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

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

Yip, C.W., Liew, C.W. and Nga, B.H. (1997). Ribosomal RNA genes of Endomyces fibuliger: isolation, sequencing and the use of the 26S rRNA gene in integrative transformation of Saccharomyces cerevisiae for efficient expression of the alpha-amylase gene of Endomyces fibuliger. *World Journal of Microbioliology and Biotechnology* 13: 103-117.

Zvauya, R., Mygohi, T. and Parawira, S. (1997). Microbial and biochemical changes occurring during production of Mavusra and Mangisi, traditional Zimbawean beverages.*Journal of plant foods Human Nutrition* 51:43-51