Studies on Certain Fermented Food Products of Nagaland

By

MS. BENDANGNARO JAMIR

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY

OF

DEPARTMENT OF BOTANY

NAGALAND UNIVERSITY, LUMAMI – 798627

NAGALAND, INDIA

2017

CONTENTS

Nagaland University

(A Central University Estd. By the Act of Parliament No. 35 of 1989)
Lumami 798 627, Nagaland, India

October 12, 2017

DECLARATION

I, **Ms. Bendangnaro Jamir**, bearing **Ph. D. Registration No. 519/2013** dated November 23, 2012 hereby declare that, the subject matter of my thesis entitled '**Studies on Certain Fermented Food Products of Nagaland**' is the record of original work done by me under the Supervision of Prof. Chitta Ranjan Deb and the contents of this thesis did not form the basis for award of any degree to me to anybody else to the best of my knowledge. The thesis has not been submitted by me for any Research Degree in any other University/Institute.

This thesis is being submitted to the Nagaland University for the Degree of **'Doctor of Philosophy in Botany**'.

(Bendangnaro Jamir)
Scholar

(Chitta Ranjan Deb)
Supervisor
Prof. Chitta Ranjan Deb
Department of Botany
Nagaland University, Lumami
Nagaland, India

(Head)
Department of Botany

HEAD
Dept. of Botany
N.U. Lumami

ACKNOWLEDGEMENT

With due respect, I would like to express my deep sense of gratitude to my esteemed teacher and Supervisor **Prof. Chitta Ranjan Deb**, Department of Botany, Nagaland University, Lumami for his persistent guidance, constant encouragement and for providing me with valuable inputs throughout my research period. He has always been extremely patient, supportive and approachable. It is definitely due to him that I could complete my research work.

I express my most sincere gratitude to Department of Biotechnology, Ministry of Science & Technology, Government of Biotechnology, New Delhi, for financial support in the form of JRF and SRF in the DBT sponsored Research programme 'Institutional Biotech Hub'.

I sincerely express my profound thanks to Honourable Vice Chancellor, Nagaland University and Dean, School of Sciences, Nagaland University for the facilities rendered to me throughout my research work.

My special thanks to the Head, Department of Botany, Nagaland University, Lumami for providing all necessary facilities for the present study.

I express my sincere gratitude to all my teachers Prof. N. S. Jamir, Prof. S. K. Chaturvedi, Dr. Talijungla, Dr. Limasenla, Dr. Sanjay Kumar, Dr. Neizo Puro, Dr. Romeo Singh and Dr. A. Paul for their support and encouragement.

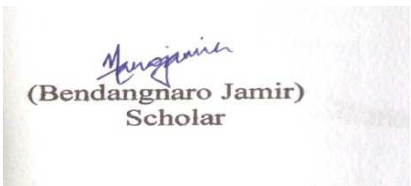
My gratitude and thanks to Salam Pradeep Singh, RA, DBT sponsored BIF centre, Nagaland University, Lumami for the help extended in different occasions.

I extend my sincere thanks to all the non-teaching staffs of Department of Botany, Nagaland University for their help and co-operation.

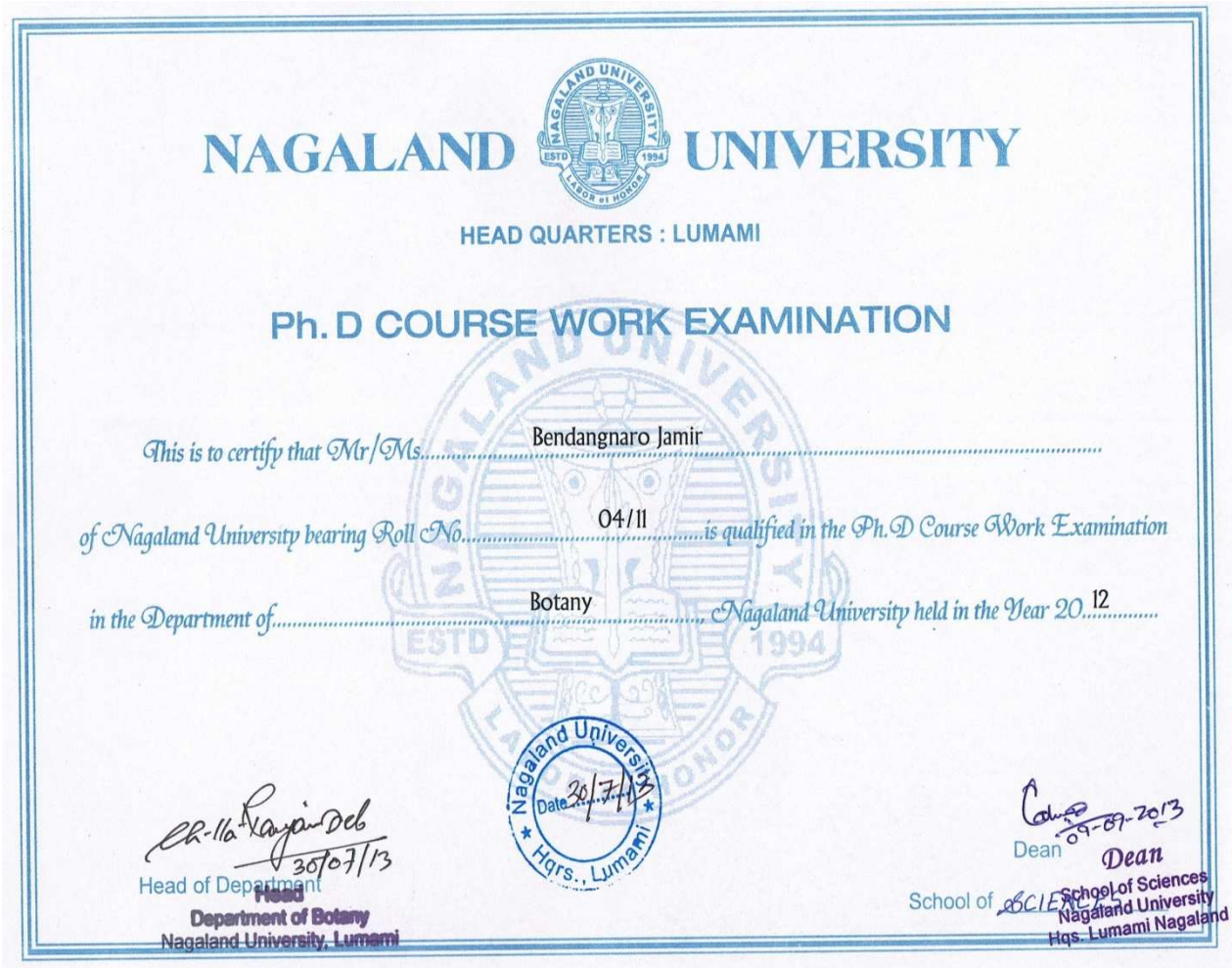
I owe my deepest gratitude to all my laboratory mates and Research Scholars of Department of Botany, Nagaland University, for their love and support in every possible way.

On my personal note, I would like to acknowledge my family members for their love, support and continuous prayers throughout my research work and for always encouraging me to give my best.

Last but not the least; I humbly give all glory and honour to God for having showered his blessings and abundant grace on me to complete my thesis successfully.



Ph. D. Course Work Certificate



NAGALAND UNIVERSITY

HEAD QUARTERS : LUMAMI

Ph. D COURSE WORK EXAMINATION

This is to certify that Mr/Ms. Bendangnaro Jamir

of Nagaland University bearing Roll No. 04/11 is qualified in the Ph.D Course Work Examination

in the Department of Botany, Nagaland University held in the Year 20 12

Nagaland University Date 30/7/13 Hqrs., Lumami

30/07/13

Head of Department

Head
Department of Botany
Nagaland University, Lumami

09-09-2013

Dean

Dean
School of Sciences
Nagaland University
Hqs. Lumami Nagaland

List of Tables

List of Figures

Abbreviations

Abbreviation	Expanded Form	Abbreviation	Expanded Form
-	Negative	**µL**	Micro litre
%	Percentage	**µM**	Micromolar
^{o}C	Degree Celsius	**mg**	Milligram
+	Positive	**DNA**	Deoxyribonucleic acid
BSA	Bovine serum albumin	**LAB**	Lactic acid Bacteria
DNSA	Dinitrosalicyclic acid	**NaCl**	Sodium chloride
H_2SO_4	Sulphuric acid	**CFU**	Colony forming unit
NaOH	Sodium hydroxide	**MRS**	de-Mann, Rogosa and Sharp
GAE	Gallic acid	**TSA**	Tryptone soya agar
QE	Quercetin	**PDA**	Potato dextrose agar
DPPH	2, 2-Diphenyl- 1-picrylhydazyl	**YMA**	Yeast malt agar
***p*H**	Potential of hydrogen	**bp**	Base pair
h	Hour	**PCR**	Polymerase chain reaction
min	Minute	**PCR-DGGE**	PCR-denaturing gradient gel electrophoresis
nm	Nanometer	**PCR-TGGE**	PCR-temperature gradient gel electrophoresis
N	Normal	**RNA**	Ribonucleic acid
g	Gram	**rRNA**	Ribosomal RNA
ml	Millilitre	**rDNA**	Ribosomal DNA
µg	Microgram	**rpm**	Revolutions per minute
pM	Picomole	**ng**	Nanomole

Chapter - 1

Introduction

Food is one of the basic human needs and it is indispensable for survival of life. Food security is a term that has been used since the last thirty years. The World Food Conference convened *by* FAO in 1974 drew the attention of the 'World Community' for the first time, to the urgent need of devising ways and means for assuring 'Food Security' to the hungry millions of the World. Food security means that food is available at all times that all persons have means of access to it; that it is nutritionally adequate in terms of quantity, quality and variety; and that it is acceptable within the given culture. Only when all these conditions are in place can a population be considered 'Food Secured' (FAO, 1996). Community food security is a strategy for ensuring secure access to adequate amounts of safe, nutritious, culturally appropriate food for everyone, produced in an environmentally sustainable way, and provided in a manner that promotes human dignity. Nutrition security is an important dimension of food security (Mahendra et al., 2003). Food is strongly connected to the culture of a community and provides it with a distinct identity. Religions and beliefs exert a strong influence on dietary habits. Ethnic

foods are defined as foods originated from a particular ethnic group of people having their own unique heritage and culture. They are generally divided into fermented foods, including beverages and non-fermented foods (Tamang, 2001; Tamang and Samuel, 2010). However, there is loss of ethnic food culture of many indigenous people due to various factors like change in climate, global economy, the process of rapid urbanization and the increasing availability of fast foods in the market (Tamang and Samuel, 2010)

Indigenous fermented foods are widely consumed as an important part of the diet of a large population of the world (Van Veen, 1957; Campbell-Platt, 1994). Foods that are invented centuries ago and even predate written historical records can be prepared by household or cottage industry using relatively simple techniques and equipment are called indigenous fermented foods (Hesseltine and Wang, 1980; Aidoo et al., 2006; Tamang, 2009).

Traditional fermented foods and beverages are popularly consumed and form an integral part of diet since early history (Aidoo et al., 2006). Traditional fermentation, smoking, drying and salting processes were developed for preservation and to improve the nutritional value of the food. These processing technologies have been modified continuously from experiences of traditional knowledge that have been passed down from generation to next (Rolle and Satin, 2002). Ethnic food fermentation process forms one of the oldest methods of food preparation and preservation which not only increases the shelf life of the food but have benefits of improving the physiochemical characteristics and nutritional quality (Nout, 2001). The diversity of these fermented products may be attributed to the heterogeneity of traditions found in the world, cultural preference, different geographical areas where they are produced and the difference in raw materials used for fermentation. They play a very important role in contributing to the livelihoods of rural people, through enhanced food security and income generation (Tamang, 2012). Indigenous fermented food products are usually prepared from locally available raw

materials of plants and animals, which are modified biochemically and organoleptically into edible product by the action of microorganisms either naturally or by adding a starter culture (Pederson, 1960; Joshi et al., 1999; Tamang and Holzapfel, 1999; Hansen, 2002). Spontaneous fermentation and lack of sterility leads to occurrence of mixed microbial populations inherent in the raw material or by microbes from the environment or preparation equipment (Yasmine, 2000). Campbell-Platt (1987) reported around 3500 global fermented foods and beverages. Tamang (2010) also reported that there might be more than 5000 varieties of common and uncommon fermented foods and alcoholic beverages being consumed in the world as food components. Today most fermented products viz. Yogurt, Kefir, Cheese, Beer, Wine, Pickles, Sauerkraut, Bread, Salami Tempeh etc. are commercially available to consumers and interest in this area is on the rise (Paramithiotis et al., 2010). However, ~90% of naturally fermented foods and alcoholic beverages are still prepared at home production under traditional conditions (Tamang et al., 2016).

Most of the traditional ethnic fermented foods are prepared by processes of solid-substrate fermentation in which the substrate is allowed to ferment naturally. On the basis of substrates, fermented foods are categorised into (1) beverages; (2) cereal products; (3) dairy products; (4) fish products; (5) fruit and vegetables; (6) legumes; and (7) meat products (Campbell-Platt, 1987).

Fermented Beverages

Fermented beverages and alcoholic drinks are culturally and socially important among the ethnic people for consumption, drinking, entertainment, customary practices, and religious purposes. These fermented beverages may be alcoholic or non-alcoholic in nature. They are usually prepared from local cereal, fruits and vegetables (Steinkraus, 1979). Examples of some indigenous fermented beverages are *Atingba* and *Yu* which are popular fermented rice wines of India, especially in Manipur state (Jeyaram et al., 2009).

To prepare *atingba* and *yu, hamei*, a natural starter culture with a flat rice-cake form is crushed into powder and mixed with cooked and cooled rice, which is then left for fermentation for 3–4 days during the summer or 5–7 days during the winter (Singh and Singh, 2006; Jeyaram et al., 2009; Tamang, 2009a). *Kanji* is an ethnic Indian strong-flavoured but mild alcoholic beverage prepared from beet and carrots by natural fermentation (Batra and Millner, 1974). *Bhaati jaanr* is the Himalayan sweet-sour, mild alcoholic food beverage paste prepared from rice and consumed as a staple food (Tamang and Thapa, 2006). *Saké* which is the national drink of Japan and is one of the most popular traditional alcoholic drinks in the world. It is prepared from rice using *koji* and is clear, pale yellow, containing 15-20% alcohol (Tamang, 2010). *Pulque* is one of the oldest alcoholic beverages prepared from the juice of the cactus (*Agave*) plant of Mexico. These beverages are mostly fermented by a combination of yeasts and LAB, with a resulting alcoholic and lactic acid fermentation (Steinkraus, 1996).

Fermented Cereal Products

Traditional fermented foods prepared from most common types of cereals, such as rice, wheat, corn or sorghum are well known in many parts of the world . In most of these products the fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi (Soni and Sandhu, 1999). Examples of cereal based fermented products are *idli* which is prepared from a blend of rice (*Oryza sativum*) and dehulled black gram (*Phaseolus mungo*) and is a very popular traditional food of India and Sri Lanka (Iyengar, 1950). Traditionally, it is prepared by first wet-soaking the rice and the black gram. After draining the excess water, both are grinded with occasional addition of water. Then the rice and the black gram batters are mixed in the ration of 2:1 with the addition of a little salt and allowed to ferment overnight. Finally, the fermented batter is steamed and taken as a popular breakfast or snack (Reddy et al., 1981). *Ogi* is an important fermented cereal from West Africa used as a traditional weaning food and nutritious meal (Oyewole,

1997). The grains usually maize but millet and sorghum can also be used, are steeped for one to three days in a container, wet-milled and then wet-sieved. The fermented *ogi* slurry is popularly consumed as breakfast porridge after cooking (Blandino et al., 2003). *Selroti* is a popular traditional product prepared by the Nepali communities of India. It is ring-shaped, rice-based bread (Yonzan and Tamang, 2009).

Fermented Dairy Products

The traditional manufacture of natural fermented milk from raw milk is spread worldwide. Milk is a source of major food components and dairy products produced from these are traditionally produced within communities (Lee, 1997). Fermented milks have a characteristic semi-solid and curdled texture, because the casein proteins in the milk are dispersed in the liquid product where an increase in viscosity occurs due to physical and chemical changes that takes place during fermentation (Wood, 1994; Gonfa et al., 2001). Examples of fermented milk products are yogurt, which is widely consumed and highly nutritious fermented milk. A general perception of the beneficial health effects associated to its consumption, led to increased production in the developed world since the 1960s. Today, yogurt is the major commercial fermented milk around the world. Yogurt can be produced from the milk of cow, buffalo, goat, sheep, yak, and other mammals, although cow's milk is predominant in industrial production (Tamime and Robinson, 2007). Traditional yogurt manufacture involved spontaneous acidification of milk (with or without boiling) at moderately high temperature (between 40°C and 50°C) (Tamime and Robinson, 2007). *Dahi* is an Indian traditional fermented dairy product, similar to the *yoghurt* of western world (Yadav et al., 2007). Fresh cow or yak's milk is boiled, cooled and allowed to ferment for 1-2 days. A small quantity of starter culture from the previously fermented *dahi* is added (Mayo et al, 2010). It is popularly consumed due to its distinctive flavour and a belief in its good nutritional and therapeutic value; is utilized in various forms in many Indian culinary preparations (Nair and Prajapati, 2003). *Kefir* is

a refreshing drink that originated on the northern slopes of the Caucasus Mountains (Koroleva, 1988). Kefir is produced by adding either a starter culture called *kefir grains* directly or a percolate of the grains to milk. Kefir grains are a mass of several different bacteria and yeasts imbedded in a complex matrix of protein and carbohydrate. The microorganisms in the kefir grains ferment the milk, and the grains can be recovered at the end of the fermentation process. Traditional home production of kefir has been joined by commercial production in many countries, and this has helped to increase the consumption of kefir and to promote its reputation as being good for health (Ismail et al., 1983).

Fermented Fish Products

Fermentation of fish has been practiced for many years in many parts of the world. Fermentation is often combined with the addition of salt or drying to reduce water activity and eliminate proteolytic and putrefying microorganisms. The process can be partial and last for several hours to several weeks (Van Veen, 1953). Dehydration, smoking, salting and fermentation are the traditional techniques for preservation of perishable fish (Beddows, 1985). There are many varieties of fermented fish products available today; examples are *suka ko maacha* which is an ethnic smoked-fish product popularly consumed by the people residing in the Himalayan regions of India (Tamang, 2010b). River fishes mostly *Schizothorax richardsonii* Gray and *Schizothorax progastus* McClelland, are used during its preparation. Fishes are degutted, washed and mixed with salt and turmeric powder. They are then dried for 7-10 days by keeping it over earthen oven (Tamang, 2010b). Fish sauce such as *nampla* in Thailand, *kecap ikan* or *bakasang* in Indonesia, *patis* in the Philippines, *nouc-mam* in Vietnam, oyster sauce, *hoi-sin* sauce, and fish and shrimp pastes such as *belacan* or *terasi* in Indonesia and Malaysia are produced and variations in the manufacture do exist between countries production. Fermentation take places for months, therefore, the quality of the raw materials is of great

importance. Fish, usually small fish like sardines, are mixed with salt and fermented to obtain a clear liquid product (Lopetcharat et al., 2001; Beddows, 1985; Olympia et al., 1992; Ijong and Ohta, 1996). *Ngari* and *Hentak* is a traditional fermented fish product of Manipur in North- East India and it reflects the food culture of the ethnic people of Manipur (Tamang, 2010b). *Ngari*, is a sun dried fermented fish product prepared with fish species of *Puntius sophore* Hamilton. *Hentak* is a ball-like thick paste prepared by fermentation of a mixture of sun-dried fish (*Esomus danricus*) powder and petioles of aroid plants (*Alocasia marcrorhiza*) (Thapa, 2002). *Hentak* is sometimes given to women during their final stages of pregnancy and to patients recovering from sickness or injury (Sarojnalini and Singh, 1988; Thapa, 2016).

Fermented Fruit and Vegetables

The development of fermentation of fruit and vegetable products started from the time ancient people started collection and storing food. Perishable and seasonal leafy vegetables, radish, cucumbers including young edible tender bamboo shoots are traditionally fermented into edible products using the indigenous knowledge of biopreservation (Watanabe et al., 2009; Cheigh and Park, 1994; Tamang et al., 2005). The preserved fermented vegetables are consumed during the long winter season when fresh leafy vegetables may not be available in plenty in the mountainous regions (Tamang et al., 2005). Well known fermented vegetable products in Asia and Europe include sauerkraut and kimchi, mainly due to their commercial importance (Lee, 1997; Kim and Chun, 2005). Traditionally, both the products are prepared by shredding cabbage and adding salt to it, but during kimchi preparation other ingredients such as radish, green onion, red pepper, garlic and ginger are added (Cheigh and Park, 1994; Lee, 1997). In Eastern Himalayan regions of India a wide range of fermented vegetable products are prepared (Tamang et al., 2005). *Gundruk* is a non-salted, fermented, and acidic vegetable product indigenous to the Himalayas. During the preparation of *gundruk*, the leaves of a

local vegetable known as *rayosag* (*Brasicca rapa* L., *Campestris* spp. (L), *Clapam* var. *crucifolia* Roxb), mustard (*Brasicca juncea* (L.) Czern.), radish (*Raphanus sativus*), or cauliflower (*Brasicca oleracea* L. var. *botrytis*) are wilted for 1-2 days and then, crushed lightly and pressed into an earthen jars or containers, made air tight and then, fermented naturally for about 15-22 days and sun dried for 3-4 days (Tamang et al., 2005; Tamang and Tamang, 2009, 2010). Pit fermentation of sinki is a unique type of biopreservation of perishable radish in the Himalayas (Tamang, 2010c). It is also practiced in the South Pacific and Ethopia (Steinkraus, 1996). *Mesu* is a non-salted fermented bamboo shoot product of the Darjeeling hills of West Bengal and Sikkim in India (Tamang and Sarkar, 1996). During its traditional preparation, young edible bamboo shoots (*Dendrocalamus sikkimensis*, *Bambusa tulda*, and *Dendrocalamus hamiltonii*) are collected, defoliated, chopped, and washed thoroughly with clean water. After draining, the chopped shoots are pressed tightly into a cylindrical bamboo container and left to ferment at ambient temperature (20-25°C) for 7-15 days (Tamang and Sarkar, 1993,1996; Tamang et al., 2008).

Fermented Legumes

Among the legumes, soybeans are mostly fermented traditionally and consumed mostly by the ethnic people of Asia. The preparation and consumption of sticky, non-salty, flavorsome fermented soybean foods are the traditional wisdom of the people from several South-East Asian countries, which have fostered a distinct food culture of the people (Nagai and Tamang. 2010). Some of the common ethnic, non-salted sticky fermented soybean foods are; *Kinema* is an alkaline soybean fermented product, which is commonly consumed by indigenous people of Nepal, the Darjeeling hills of West Bengal, and Sikkim, in India. It is a product similar to Japanese *natto*, Korean *chungkukjang*, and Chinese *schuidouchi* (Sarkar et al., 1994; Moktan et al., 2008). To prepare *kinema* traditionally, yellow seeded soybeans are washed, soaked overnight in water , cooked by

boiling until softened, crushed lightly to grits, wrapped in fresh fern leaves and sackcloth and left to ferment for 1-3 days (Sarkar and Tamang, 1995; Tamang and Nikkuri, 1998; Dahal et al., 2005; Moktan et al., 2008). Instead of fern leaves, *Ficus* or banana leaves are also used as a wrapping material (Sarkar and Tamang, 1995; Sarkar et al., 1998; Singh et al., 2007a; Moktan et al., 2008). *Tempeh*, or *tempe kedelee*, which originated in Indonesia, is made by the fermentation of soybeans. *Tempeh* is used as a main dish in Indonesia. *Tempe* has an overwhelming advantage in terms of odour over other non-salted fermented soybeans. The general process involves using soybeans that are soaked in water, the seed coat is removed, and the soybeans are drained and cooked, and then drained and cooled. They are then inoculated with spores of *Rhizopus oligosporus*, packed into trays, and incubated 20-24 h at 30-38°C. Matured *tempeh* is ready for consumption either raw or cooked (Steinkraus et al., 1960; Batra and Millner, 1974). Fermented soybean pastes are known as *miso* in Japan, *chiang* in China, *jang* or *doenjang* in Korea, *taoco* in Indonesia, and *tao chieo* in Thailand. In addition to soybeans and salt, most of these products contain cereals such as rice or barley (Minamiyama and Okada, 2003). *Bikalga, dawadawa, iru, mbodi, ntoba* and *soumbala* are the ethnic nonsalted fermented locust bean (*Parkia biglobosa*) foods of Africa (Amoa-Awua et al., 2006, Azokpota et al., 2006, Meerak et al., 2008, Ouoba et al., 2010).

Meat Based Fermented Products

Meat fermentation is a low energy, biological acidulation, preservation method which results in unique and distinctive meat properties such as flavour and palatability, colour, microbiological safety, tenderness, and a host of other desirable attributes of this specialized meat item (Campbell-Platt and Cook, 1995). The traditional methods employed for prevention of microbial spoilage are still in use, though with a different meaning in the various products. These methods comprise reduction of water activity (drying, salting) and *p*H (fermentation, acidification), smoking, storage at refrigeration or

freezing temperatures, and use of curing aids (nitrite and nitrate). Commonly, these methods act together in different combinations, building up hurdles against microbial growth (Rai et al., 2009).Today in various parts of the world, a large number of different types of fermented sausage exist. This very high consumption of fermented meats is an indication that such products have a long tradition of being safe (Lee, 1997). Examples include sausages, where, product is chopped, mixed with salt, sometimes nitrite and/or nitrate, sugar, usually starter cultures, and seasoning, stuffed into casings. It is sometimes smoked which aids preservation and inhibits surface mold growth. Meat fermented products undergoes lactic acid fermentation, however, yeast and mycelia fungi can also be present, especially in traditionally prepared sausages (Lücke, 1985; Lee, 1997; Papavergou, 2011).

Fermentation

The World Health Organisation food safety unit has given high priority to the research area of fermentation as a technique for preparation/storage of food. The term fermentation is derived from the Latin word '*fervere'* meaning "to boil", since the bubbling and foaming of early fermenting beverages closely resembles to boiling. It is the chemical transformation of organic substances into simpler compounds by the action of enzymes, the complex organic catalysts which are produced by microorganisms such as molds, yeasts, or bacteria (Petchkongkaew, 2007), and due to the enzymatic activity various by-products are formed (Bisen et al., 2012). Technically, Campbell-Platt (1987) has defined fermented foods as those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. The study of fermentation is called '**Zymology'** and the first zymologist was Louis Pasteur (1861 AD), who was the first to educate the world that yeast is responsible for fermentation.

Natural fermentation precedes human history. Since ancient times, however, human have been controlling the fermentation process. Wang and Hasseltine (1979) noted that probably the first fermentation was discovered accidentally. There is strong evidence that fermented beverages were the oldest known fermented product, and traces back to at least 2000-4000 BC followed by the Egyptians and Sumerians (1). Ancient Indian alcoholic beverage *Soma* is mentioned in the *Rigvedas* (ca. 1500 BC) (Prajapati and Nair, 2003). Fermented milk, meats and vegetables have been described to date back to 6000 BC in the civilization of the Fertile Crescent in the Middle East (Caplice and Fitzgerald, 1999). The art of fermentation in the Indian sub-continent appears to have predated the Indus Valley civilization (Padmaja and George, 1999). Fermentation became popular with the dawn of civilization because it not only preserved food but also gives a variety of tastes, forms and other sensory sensations.

Fermentation processes for thousands of years were carried out without understanding the microbial mechanisms until 1907 when the famous Nobel prize-winning Russian Bacteriologist, Elie Metchnikoff, first considered the possible benefit to good health from fermented foods (Mehta et al., 2012). Since the late 1920s scientists have continued to investigate the possible benefit to the health of bacteria. In 1935, certain strains of *Lactobacillus acidophilus* were found to be very active when implanted in the human digestive tract. Further research throughout the last forty years has found more and more health benefits using friendly bacteria.

Fermented foods harbour diverse microorganisms from the environment that include filamentous molds, yeasts and bacteria, which may be indigenously present on the substrate, or added as a starter culture (Blandino et al., 2003), or may be present in or on the ingredients and utensils, or in the environment - are selected through adaptation to the substrate and by adjusting the fermentation conditions (Soni and Sandhu, 1990). In back-slopping, a part of a precious batch of a fermented product is used to inoculate the new

batch. This procedure produces a higher initial number of beneficial microorganisms than that found in the raw material and ensures a faster and more reliable fermentation than that which occurs in spontaneous fermentation (Josephsen and Jespersen 2004). During fermentation, carbohydrates are oxidised by microbes aerobically or anaerobically. The end-products produced mainly include lactic acid, but also carbon dioxide and alcohol (Ross et al., 2002). These microbes may also produce other organic acids such as acetic, propionic, fomic and butyric acids, as well as enzymes, bacteriocins, aroma compounds and exopolysaccharides (Caplice and Fitzgerald, 1999; Leroy and De Vuyst, 2004). This results in the production of a safe fermented product with reduced *p*H having unique sensory characteristics and partially contains some carbohydrates so is therefore nutritionally beneficial to the human diet (Caplice and Fitzgerald, 1999).

There are four main fermentation processes, namely, alcoholic, lactic acid, acetic acid, and alkaline fermentation (Soni and Sandhu, 1990; Joshi and Pandey, 1999; McKay et al., 2011; Sarkar and Nout, 2014).

Alcoholic Fermentation

Alcoholic fermentation is one of the most important and the oldest process (Steinkraus, 1979; Amoa-Awua, 2006) involving production of mainly ethanol and carbon dioxide. Products of alcoholic fermentations are beer, wine and bread. These are generally yeast fermentations, but can also involve yeast-like molds, such as *Amylomyces rouxii*, and mold-like yeasts such as *Endomycopsis* and sometimes bacteria such as *Zymomonas mobilis* (Steinkraus, 2002). *Sake*, a fermented rice wine of Japan, a combination of a mold (*Aspergillus oryzae*) and yeast (*Saccharomyces cerevisiae*) are involved during its production (Steinkraus, 1983).

Lactic-acid Fermentation

Lactic acid bacteria are gram-positive, catalase-negative bacteria that produce large amounts of lactic acid. Traditional uses of many Lactic acid bacteria (LAB) as fermenting agents for foods are considered safe for the general population. Lactic acid bacteria perform an essential role in the preservation and production of wholesome fermented foods by lowering the *p*H rapidly to a point where other competing microorganisms are no longer able to grow (Kumar et al., 2013). The most ancient lactic acid fermentation is fermented milk product, as the lactic acid bacteria present in the milk ferment milk sugar, lactose to lactic acid (Steinkraus, 2002). The production of cheese is also a typical lactic fermentation of milk, carried out by using a suitable starter culture of lactic acid bacteria (Cogan and Hill, 1993; Cogan and Accolas, 1996). Fermentation of vegetables/fish/shrimp is preserved around the world by lactic acid fermentation (Steinkraus, 1983; 1996). Mostly species of *Lactobacillus* and *Pediococcus*, followed by *Leuconostoc*, *Weisella*, *Tetragenococcus*, and *Lactococcus* (Watanabe et al., 2009, Savadogo et al., 2011) have been isolated from various fermented vegetable foods of the world.

Acetic Fermentation

Fermentation involving the production of acetic acid which yields foods or condiments that are generally considered as safe, as acetic acid is either bacteriostatic or bactericidal, depending upon the concentration employed. After alcohol production, when products are not maintained at an anaerobic condition, bacteria belonging to the genus *Acetobacter* present in the environment oxidize portions of the ethanol to acetic acid/vinegar (Steinkraus, 2002). Vinegar is a highly acceptable condiment used in pickling and preserving cucumbers and other vegetables (Connor and Allgier, 1976).

Alkaline Fermentation

Fermented foods involving highly alkaline fermentations are generally considered as safe. The essential microorganisms are *Bacillus subtilis* and related bacilli. Due to enzymatic hydrolysis of the proteins into peptides and amino acids, ammonia is released which increases the *p*H to as high as 8.0 or higher. The combination of high *p*H and free ammonia along with incubation at high temperature above 40^{o}C make it difficult for other spoilage microorganisms to grow. Thus, the products are quite safe even though manufactured in an unhealthy environment (Steinkraus, 2002; Sarkar and Tamang, 1995). Most of the fermented soybean products undergo alkaline fermentation and have reported the presence of *Bacillus* species (Tamang et al., 2002; Jeyaram et al., 2008; Kwon et al., 2009). Bacillus species have also been isolated from ethnic non-salted fermented locust bean foods of Africa such as *dawadawa*, *iru* and *soumbala* (Azokpota et al., 2006; Ouoba et al., 2010).

Benefits of Fermented Foods

Ethnic fermented food products have been associated with good health and longevity. Fermented foods may also contribute in reducing hunger by adding nutritional value to food and increase the bioavailability of nutrients (Tamang, 2011; Nah and Chau, 2010). Fermentation ensures not only increased shelf life and microbiological safety of a food but also makes food more digestible. Changes in the fermented product depends on various factors, such as the availability of nutrients and nutrients precursors in the starting materials, the metabolic capabilities of the starting materials and the metabolic abilities of the fermentative microorganisms (Adams and Nout, 2001). Indigenous fermentation technologies were based on experiences accumulated by consecutive generations of food producers, through trial and error. Only recently has science and technology started to contribute to a better understanding of the underlying principles of fermentation processes and of the requirements for quality and safety (Tamng et al., 2016a).

Biopreservation and Improvement in Food quality

Fermentation is one of the oldest processing techniques to extend the shelf life of perishable food and was particularly important before refrigeration (Hesseltine and Wang, 1980). The preservative action of microorganisms is attributed to the production of many organic acids such as lactic, acetic and propionic acids, which provides an acidic environment unfavourable for the growth of many pathogenic and spoilage microbes. Due to low *p*H (3.3-3.8) and high acid content (1.0-1.3%), fermented products like *gundruk* and *sinki* after sun drying, can be preserved without refrigeration for more than two years without addition of any synthetic preservative (Tamang, 2010d). In addition to acids, microorganisms produce a range of other microbial metabolites such as carbon dioxide and ethanol from the hetrofermentative pathway, hydrogen peroxide produced during anaerobic growth by oxidising flavoproteins and diacetyl antifungal compounds such as fatty acids or phenyllactic acid, bacteriocins, and antibiotics such as reutericyclin produced by bacterial species and strains (Settanni and Corsetti, 2008). *Kimchi* has high antimicrobial activity against *Listeria monocytogenes*, *Staphylococcus aureus*, *E.coli* and *Salmonella typhimurium* (Kim and Park, 1995; Khan and Kang, 2016). Fermentation helps in the development of diversity of flavours, aromas and textures in food substrates and, therefore, adds variety to the diet and makes the food more palatable and, ultimately, more popular than the unfermented food (Caplice and Fitzerald, 1999; Blandino et al., 2003; Chelule et al., 2010). The biological transformation of a bland vegetable protein into meat-flavoured amino acid sauce and paste by mold fermentation is common in the Japanese *miso* and *shoyu*, the Chinese soy sauce (Steinkraus, 1996). The action of microorganisms during the preparation of fermented foods has been shown to improve the digestibility of some dietary nutrients through destruction of anti-nutritional factors, such as phytic acid, trypsin inhibitors, nonstarch polysaccharides, oligosaccharides, lectins, and saponins. Moreover, microorganisms contain certain enzymes that are

incapable of being synthesized by humans. Thus, these enzymes can hydrolyze complex food components into simple units, which are then readily digested or absorbed by humans. Milk a highly nutritious beverage, is made more acceptable to lactose-intolerant individuals through milk fermentation due to conversion of lactose to simple sugars-glucose and galactose. Microbes also produce various enzymes capable of breaking down cellulose in plant foods into sugars, making it more digestible (Sathe and Mandal, 2016).

Enhancement of Nutritional Quality

Nutritional quality of food can be enhanced by fermentation, with essential amino acids, vitamins, and various bioactive compounds (Sarkar et al., 1998). Food fermentations that raise the protein content or improve the balance of essential amino acids or their availability, as well as increase in vitamins such as thiamine, riboflavin, niacin or folic acid have profound effect on the health of the consumers (Steinkraus, 2002). Fermentation is a cost effective way to enrich food with essential amino acids and vitamins which can help prevent malnutrition (Holzapfel, 2002; Motarjemi, 2002). In *tempe*, the levels of niacin, nicotinamide, riboflavin, and pyridoxine are increased by *Rhizopus oligosporus*, whereas cyanocobalamin or vitamin B12 is synthesized by non-pathogenic strains of *Klebsiella pneumonia* and *Citrobacter freundii* during fermentation (Liem et al., 1977). *Bacillus* strains from fermenting locust beans in Africa have been found to produce glutamic acid and extracellular proteinases (Ogbadu et al., 1990). Different enzymes like amylase, glucoamylase, protease, lipase, etc. (Tamang and Nikkuni, 1996; Thapa, 2001; Tamang, 2010) are produced in traditional fermented foods that are beneficial for health, and so the fermented products serve as a source of enzymes. Proteins are partially hydrolysed into free amino acids, which contribute as synergists to the stability of fermented products against oxidation (Erbas et al., 2005). Studies on antioxidant properties of fermented soybean have shown increase in polyphenols (isoflavones, phenolic acids and flavanols) are responsible for increase in antioxidant

property (Sanjukta and Rai, 2016). Alkaline fermented foods like *Kinema* have also been associated with antioxidants property (Sarkar and Nout, 2014; Sanjukta et al., 2015). Increase in total phenol content, which is one of the indicators of antioxidant activity, has been reported in *Chungkokjang*, a Korean fermented soybean food (Shon et al., 2007) and in *douche*, a Chinese fermented soybean food (Wang et al., 2007).

Elimination of Toxins

Agricultural products, food and animal feeds can be contaminated by toxins and lead to various diseases in humans and livestock (Hasan et al., 2014). Food and feeds are often contaminated with a number of toxins, either naturally or through contamination with microorganisms (Chelule et al., 2010). Mycotoxins are toxic secondary metabolites that are produced, for the most part, by fungi belonging to the *Aspergillus, Penicillium*, and *Fusarium* spp. (Sweeney and Dobson, 1998). Microorganisms present in the fermented food products such as *Saccaharomyces cerevisiae* has the ability to degrade mycotoxins to less non-toxic products, by inhibiting the absorption of mycotoxins in the gastrointestinal tract (Hasan et al., 2014). Aflatoxin, found in peanut and cereal grains are reduced during the production of *ontjom*, by the combination of mold *Neurospora* and *Rhizopus oligosporus* (Steinkraus, 2002). Lactic acid bacteria in fermentation also detoxify toxins and preserve the nutritive value and flavour of foods (Chelule et al., 2010; Ari et al., 2012). LAB fermentation has successfully detoxifed cassava toxins (cyanogens) by fermentation (Caplice and Fitzgerald, 1999; Chelule et al., 2010).

Health Benefits

Ethnic fermented foods are considered to be a good source of food to meet up hunger and also as curative (Shin and Jeong, 2015). Fermented foods often include Lactic acid bacteria strains with probiotic properties. The FAO/WHO defined probiotic as ‘live microorganisms’ which, when administered in adequate amounts, confer health benefits on the host (Giraffa et al., 2010). The health benefits of probiotics for humans include

control of inflammatory bowel diseases, balanced immune system, lowering of cholesterol level; improve lactose tolerance, reduction in risk of colon cancer and so on. The probiotic bacteris used in commercial products today are mainly members of the genera Lactobacillus and Bifidobacterium (Hasan et al., 2014). Fermented food products from lactic acid bacteria provide the human body with a variety of important nutritional and therapeutic benefits, including antimutagenic and anticarcinogenic activities (Lee and Lucey, 2004). Consumption of fermented milks or probiotic bacteria lowers the risk of heart diseases (Huth and Park, 2012). Indian *dahi*, has anti-carcinogenic property (Mohania et al., 2014). Administration of some strains of *Lactobacillus* improves the inflammatory bowel disease, paucities and ulcerative colitis (Orel and Trop, 2014). In fermented soybean products such as *natto* and *tempe*, antihypertensive properties were observed (Wu and Ding, 2001; Sanjukta et al., 2017). Studies revealed that the bacterial strains of *Bacillus subtilis* CSY191 isolated from *doenjang*, produced a surfactin like compound which posses anticancer property (Lee et al., 2012). Consumption of *chungkokjang* on a daily basis may increase the insulin resistivity thus controlling diabetics (Tolhurst et al., 2012; Thapa and Tamang, 2015). Several health benefits of *kimchi* have been reported such as prevention of colon cancer, reduction in cholesterol level; improve cardiovascular diseases and also as anti-stress property (Lee and Lee, 2009; Park et al., 2014).

Risk of Fermented Foods

Most of the indigenous fermented foods are still prepared at the household level, by the womenfolk (Singh et al., 2007). Therefore, there is high risk of contamination from unhygienic handling of the substrates, utensils and unclean hands during the processing method. Storage of these fermented products is also one of the causes of contamination. The risk is increased when plastic container is used instead of the old-fashioned and traditional method. Spoilage of fermented products by many prevalent

food-borne bacterial pathogens during the production of such foods has also been described (Farnworth, 2003). The productions of biogenic amines during the fermentation process, which are organic compounds produced by the microbial decarboxylation of their precursor amines, are considered as unhealthy and has adverse effect on human health (Tamang et al., 2016). The fermented products need to be prepared under good sanitary and hygienic conditions. (Motarjemi et al., 1993) and efforts be must made to avoid these foods being a source of contamination.

Fermented Foods and Beverages of North-East, India

North-eastern region of India is characterized by a diverse population of people with different ethnic background. The knowledge and utilization of local plant depends on the ethnic group they belong to (Singh et al., 2007). This region comprises the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. It lies between 21^{o}34´ N to 29^{o}50´ N Latitude and 87^{o}32´ E to 97^{o}52´ E Longitude and covers an area of ca 262060 sq km (Mao et al., 2008). It has the richest reservoir of plant diversity in India and is one of the 'biodiversity hotspots' of the world. Agriculture is the main occupation of the people and they depend on 'Shifting' or '*Jhum*' cultivation systems and forest based foods, which ensure a range of ethnic foods rich in nutrition compatible to culture and ethnicity of different tribes (Dutta abd Dutta, 2004). Individual ethnic tribes have developed their own socio-cultural patterns and unique food habits. Every community has developed its own fermentation techniques for transformation of different raw materials of plant and animal origin (Sekar and Mariappan, 2007).

Most commonly used raw materials by the local people for the production of the various traditional fermented foods and beverages are cereals, leafy vegetables, bamboo shoots, fishes and meat. Tamang and Sarkar (1988) studied on the indigenous fermented food and beverages of Darjeeling hills and Sikkim. Some major documented fermented

products were *Kinema* (soybean); *Mesu* (bamboo shoot); *Gundruk, Sinki* and *Khalpi* (vegetables); *Chhurpi* (fermented cow or yak milk); *Selroti* (rice based); *Bhaati jaanr* (alcoholic beverage); *Suka ko maacha* (fish based). Agrahar-Murugkar and Subbulakshmi (2006) studied the preparative techniques as well as the nutritional value of fermented foods of the *Khasi* tribes of Meghalaya. Fermented soybean product *tungrymbai* contained high amounts of protein, fat, fiber, carotene and folic acid; fermented bamboo shoot *lungsiej* was reported to be more nutritious than its counterpart in terms of protein and iron; fermented fish product *tungtap* was reported to be good source of protein, calcium, phosphorus, sodium and potassium. Tiwari and Mahanta (2007) studied the ethnological significance of traditional fermented products prepared by some tribes of Arunachal Pradesh. The fermented products studied were; *Churapi* and *Churkham* (fermented yak milk); *Pikey Pila* and *Tapyo* (vegetable based); *Apong* and *Emong* (beverages); *Bamboo tenga* (bamboo shoot based). Mao and Odyuo (2007) documented some of the fermented foods of the *Naga* tribes of North-East India namely *Anishi* (vegetable based); *Axone* (soybean based); fermented bamboo shoot, fish, crab and animal fats. Jeyaram et al., (2009) documented the traditional preparation processes of fermented foods of Manipur, such as *Hawaijar* (soybean based); *Soibum/Soijim* and *Soidon* (bamboo shoot based); *Ngari* and *Hentak* (fish based); *Ziang sang* and *Dui* (mustard leaf based) and *Atingba* and fruit wines (fermented beverages). Chakrabarty et al. (2009) did studies on the utilization of substrate by the tribes of North Cachar Hills District of Assam, for the preparation of various traditional fermented foods and beverages. Some of the documented fermented products were *bekanthu* (soybean based); *miya* (bamboo shoot based); *saphak* and *satu* (fermented pork fat); *judima, zunak, dekuijao* and *juharo* (fermented beverages). Das et al. (2012) documented the preparation methods of some of the fermented foods and beverages of North-East India. Chakrabarty et al. (2014) studied the preparative process, microbiology and the nutritional value of some ethnic fermented foods and beverages of North Cachar Hills district of Assam.

Documented fermented products were *Tuaithur* (wet) and *Tuairoi* (dry) (bamboo shoot); *Honoheingrain* (fermented pork/boar meat); *Humao* (amylolytic dough starter); *Judima* (fermented beverage). Microorganisms isolated were: lactic acid bacteria represented by 5 genera- *Lactobacillus, Leuconostoc, Pediococcus, Enterococcus and Lactococcus;* bacteria belonging to *Bacillus* sp.; *Staphylococcus aureus* and *Micrococcus* sp.; yeasts- *Debaryomyces hansenii* and *Saccharomyces cerevisae*; moulds- *Mucor* and *Rhizopus.* Jamir and Deb (2014) documented some of the fermented foods and beverages of Nagaland, India. They were classified based on the raw material used for its production i.e, *Zutho* (cereal based alcoholic beverage); *Axone/Akhuni* (soybean based); *Anishi, Hungrii* and *Tsutuocie* (vegetable based); *Bastanga* (bamboo shoot based); *Jangpangngatsu, Jang kap* and fermented pork fat (meat based); fruit based beverages. Uchoi et al. (2015) studied the various traditional cured foods of certain tribes of Tripura, their methods of preparation, uses, nutritional and medicinal values. Reported fermented products were *Moiya Koshak, Melye Amiley* and *Moiya Pangsung* (bamboo shoot); *Batema* (fermented elephant yam); *Amlai Ntoi, Kosoi* and *Bikang* (fruit and vegetable); *Bochu-mba* (Bombax ceiba flower); *Lungi* or *Gora* (fermented beverage); *Shidal* and *Lona ilish* (fermented fish). Tamang (2015) documented the various fermented soybean products of the tribes of Northeast India viz. Sikkim, Manipur, Meghalaya, Nagaland, Mizoram and Arunachal Pradesh. He reported that *Bacillus subtilis* was the most dominant functional bacterium in all the naturally fermented soybean foods of these regions.

Researches on some of the fermented foods and beverages of North-eastern region have been extensively done, whereas some are still to be explored and studied. *Kinema* and *Hawaijar* are the most studied fermented soybean products (Jeyaram et al., 2009). The microbial diversity and the proximate composition of *Kinema* was studied and recovered the heat resistant spore-forming bacterium *Bacillus subtilis*, lactic acid bacteria

such as *Enterococcus faecium* and a few types of yeast, *Candida parapsilosos* and *Geotrichum candidum*. *Kinema* was reported to be more nutritious than the raw soybeans (Sarkar et al., 1994; Tamang, 2003). Moktan et al. (2008) studied the antioxidant activity of *Kinema* and reported high level of antioxidant activity compared to cooked non-fermented soybean, rendering it to be a functional food capable of alleviating oxidative stress. Jeyaram et al. (2008) studied the dominant microorganisms associated with *Hawaijar* and reported three major phylogenic groups, *Bacillus subtilis* group comprising *B. subtilis* and *B. licheniformis*, *B. cereus* group and *Staphylococcus* spp. group comprising *S. aureus* and *S. sciuri*. Premarani and Chhetry (2011) studied the nutritional value associated with *Hawaijar*, the fermented soybean food product of the people of Manipur, India. Sohliya et al. (2009) did studies *on Tungrymbai*, a traditional fermented soybean of the ethnic tribes of Meghalaya and reported the presence of various species of lactic acid bacteria, yeasts and spore forming bacteria. Chettri and Tamang (2015) also did studies on *Tungrymbai* and *bekang* which are naturally fermented soybean foods of Meghalaya and Mizoram. They reported that the most dominant bacterium in both products was *Bacillus subtilis* in addition to the presence of various other *Bacillus* sp. groups.

Teramoto et al. (2002) studied the fermented alcoholic beverage of the Naga tribe, called *Zutho* and identified a fermentation yeast strain of *Saccharomyces cerevisiae* which was found to be suitable brewing yeast for ethanol fermentation. Tamang and Thapa (2006) studied the traditional fermented beverage *Bhaati Jaanr*, prepared from steamed glutinous rice in the East Himalayan regions of Nepal, India and Bhutan. It was revealed that *Saccharomycopsis fibuligera* and *Rhizopus* sp. play important role in the fermentation process. Tanti et al. (2010) studied the production of household liquors by the North-Eastern people of India. His study highlighted the potentials of the ethnobotanical research and the need for documentation of traditional knowledge

pertaining to the production of alcohol. Shrivastava et al. (2012) studied the preparation of alcoholic beverage followed by the different tribes of Arunachal Pradesh and their relation to the tribal lifestyle of the state. Arjun et al. (2014) studied on the method of preparation and biochemical analysis of local tribal wine *Judima:* an indegenous alcohol used by Dimasa tribe of North Cachhar Hills District of Assam, India. They reported *Judima* contains good amounts of protein, carbohydrate and free amino acids with high antioxidant activity.

Tamang and Sarkar (1996) studied on the microbiology of *mesu*, a traditional fermented bamboo shoot product of Sikkim. They reported the presence *Lactobacillus plantarum, L. brevis* and *Pediococcus pentosaceus.* Sarangthem and Singh (2003) reported the presence of bacterial group of *Bacillus* sp. in the fermented bamboo shoot product of Manipur. Tamang and Tamang (2009a) isolated and identified the predominant LAB namely *Lactobacillus plantarum, L. brevis, L. casei, L. fermentum, Lactococcus lactis* and *Tetragenococcus halophiles* from the indigenous fermented bamboo shoots of Arunachal Pradesh. Choudhury et al. (2012) studied the biochemistry and microbiology associated with the traditional fermented bamboo shoots. Satya et al. (2012) studied the nutritional value of fermented products made of different species of bamboo shoots and the effect of processing methods on its nutritive quality. Sonar et al. (2015) studied the nutritional and functional profile of traditional fermented bamboo shoot products of Arunachal Pradesh and Manipur. They reported the products to be highly acidic with high crude fiber content and low fat content. All products exhibited significant radical scavenging activity and α-glucosidas inhibitory activity.

Tamang et al. (2005) studied on the microbiology of *gundruk, sinki, khalpi* and *inziangsang,* which are traditionally fermented vegetable products. The phenotypic characterization followed by genotyping resulted in the identification of LAB namely *Lactobacillus brevis, Lactobacillus plantarum, Pediococcus pentosaceus, Pediococcus*

acidilactici and *Leuconoctoc fallax*. Tamang and Tamang (2009) did studies on the traditional fermented vegetables and bamboo shoots of the Northeast India and reported them to be good source of food supplement.

Majumdar et al. (2010) did studies on the traditional fermented fish product *Lona ilish* of the Northeast India. Its chemical composition and microbial composition were studied. Thapa et al. (2004) studied the microbial diversity in *ngari, hentak* and *tungtap*, which are traditional fermented fish products of North-East India. Lactic acid bacteria of the group *Lactobacillus* were identified; endospore-forming rods were identified as *Bacillus subtilis* and *Bacillus pumilus*, aerobic coccal strains were identified as *Micrococcus*; yeasts were identified as *Candida* and *Saccharomycopsis*.

Tamang et al. (2012) studied the diverse microorganisms associated with fermentation and production of ethnic foods and alcoholic drinks. It was demonstrated that the functional microorganisms present in the ethnic foods of Northeast have many biological functions enhancing the health promoting benefits, bio-preservation, protective properties and therapeutic values. However, much work has still not been done on the fermented food products of Nagaland and there is still vast scope for studying on the traditional fermented foods and beverages of the different *Naga* tribes (Mao and Odyuo, 2007). Such study has been taken up to preserve the traditional knowledge by documenting the various fermented foods used by the different tribes of Nagaland and the processes involved during its preparation. Traditional fermented products in Nagaland are still prepared at the household level and at a time where food security is one of the major challenges faced today, the need for better food quality which is nutritionally beneficial to the population has become the need of the hour. Thus, there is a need for better quality control of the product for it to be recognised and popularized.

Based on the above background fermented food products can be consumed not only as a food source but also as curative. There is little scientific report on the nutritional and

antioxidant activity of fermented foods of Nagaland and the various microorganisms associated with them. Therefore, this present investigation was undertaken with the following objectives:

1. Survey and documentation of important fermented food products consumed by the different ethnic tribes of Nagaland.
2. To replicate the same scientifically in the laboratory.
3. To isolate the microorganisms associated with the processed fermented foods and make pure culture from them.
4. Nutritional analysis of the selected fermented food products and comparison with the raw materials.

Chapter - 2

Documentation of Fermented Foods of Nagaland

Nagaland, one of the North-Eastern states of India lies between 13°37´09˝ N Longitude- 123°10´53˝ E Latitude and is surrounded by different states of India; the hills of Manipur, North Cachar and Mikir hills, Lakhimpur, Sabsagar and Nowgong of Assam, two districts of Arunachal Pradesh (Changlan and Tirap) and across the border of Myanmar. *Nagas* are of the Mongolian race and forms one of the largest tribal community comprising of 16 different tribes of which 14 are recognised, viz. *Angami, Ao, Chakhesang, Chang, Kongyak, Khiamungan, Sema, Rengma, Lotha, Sangtam, Phom, Zeliang, Pochury* and *Yimchunger*. The state is a part of one of the biodiversity hotspots of India, having rich forest resources. Agriculture is considered as the main source of livelihood of the people of Nagaland, with over 70% of its population dependent on it for food and economic stability. The diverse agro-climatic conditions, varied soil types and abundant rainfall prevailing in the state enable the cultivation of several agricultural

crops. The people of Nagaland practice the '*Jhum'* and terrace cultivation leading to preservation of range of local crops, ethnic vegetables and fruits for food and nutritional security. Rice is the dominant crop and also the staple diet of the people. Principal crops are yam, millet, maize, pulses, potato and sugarcane. Vegetable crops are melon, cucumber, spinach leaf, mustard, onion, chillies, carrots, tomato; brinjal etc. Naga people have rich reserve of traditional knowledge. This traditional knowledge includes practices which play a fundamental role in people's livelihood, health, food and food habits. Traditional knowledge has great potential value for sustainable development, so it is necessary to preserve indigenous knowledge for the benefit of future generations (Dixit and Goyal, 2011).

Indigenous fermented foods have been prepared and consumed for thousands of years, and strongly linked to culture; traditions and reveal the intellectual richness of indigenous people of the country in terms of their ability to prepare microbial products for varied purposes in addition to food and beverages (Sekar and Mariappan, 2007). Traditional fermented foods in Nagaland are popularly consumed at a large scale. It forms a part of the daily diet and adds flavour to otherwise bland curries. The women folks of Naga villages, process various raw materials such as local vegetables, fruits, animal fats and beverages (Mao and Odyuo, 2007; Singh et al., 2007). These fermented foods are still prepared at the household level and usually natural or spontaneous fermentation occurs during its production. Fermentation results as a consequence of the competitive activities of different microorganisms and strains best adapted and with higher growth rates usually dominate (Aka et al., 2014). Due to the diversity of ethnic groups in Nagaland, there are also various fermented food products produced by each ethnic group. Each ethnic group has its own method of fermenting food materials for the purpose of preservation, taste, and nutritional enhancement and has been carrying this tradition from time immemorial

(Jamir and Deb, 2014). However, due to influence of global commercialization and socio-economic transformation, the preparation of some lesser known fermented foods are fast declining (Narzary et al., 2016) and the details of the preparation steps of the various fermented food products have not been systematically studied and recorded. It is important to document the process, quantify the ingredients and identify the key conditions for a successful fermentation in order to replicate the process under standardised conditions and ultimately at industrial level. Thus, this chapter deals with documentation of traditional processing method of some of the major traditional fermented food products used by some of the major *Naga* tribes of Nagaland.

Methodology

Field survey was conducted in different parts of Nagaland and documentation study was carried out from different villages and markets in and around Nagaland. Since documentation of the traditional knowledge associated with preparation of the indigenous fermented foods was done on region and tribe specificity, the following regions were surveyed for collection of information; Kohima, Tseminyu, Wokha, Mokokchung and Zunheboto. Information on the step by step method for production of the various traditional fermented products was collected through personal discussion and participation with the local people. Fermented foods are categorized as follows:

(1) Cereal and legume based fermented product.

(2) Vegetable based fermented product.

(3) Bamboo shoot based fermented product.

(4) Meat based fermented product.

(5) Fruit based fermented beverages.

Documentation/Results

1. Cereal and legume Based Fermented Product

(i) *Zutho*

'*Zutho*' (rice beer) is a traditional alcoholic beverage prepared from rice (*Oryza sativa* L.), named according to the *Angami Naga* dialect. It is prepared in two parts.

A. Preparation of malt: Malt is prepared by soaking the unhulled rice grains in water for about 2-3 h and allowed to germinate. The germinated grains are then spread on bamboo mats and left to dry in the sun. Using traditional mortar and pestle, the malt is pounded into powder.

B. Preparation of *Zutho:* Polished rice grains are first washed and soaked in water for 30 min, after which the excess water is drained off. It is then spread over bamboo mats and allowed to air dry. It is pounded into powder and hot boiling water is added to the rice powder bit by bit and kept aside for some time to allow it to cool down. The powder of malt and polished rice grain powder are mixed together in the ratio of 3:7. After proper mixing, it is kept at room temperature and allowed to ferment for about 4-5 days (**Figure 2.1, 2.2**). The first stock in its pure form is called '*Thutshe*' and after it is diluted with some amount of water it is called *'Zutho*'.

Socio-economic importance: *Zutho* is consumed as a popular alcoholic beverage in Nagaland. These are generally made during cultural festivals and marriages. The flow chart of preparation is shown below:

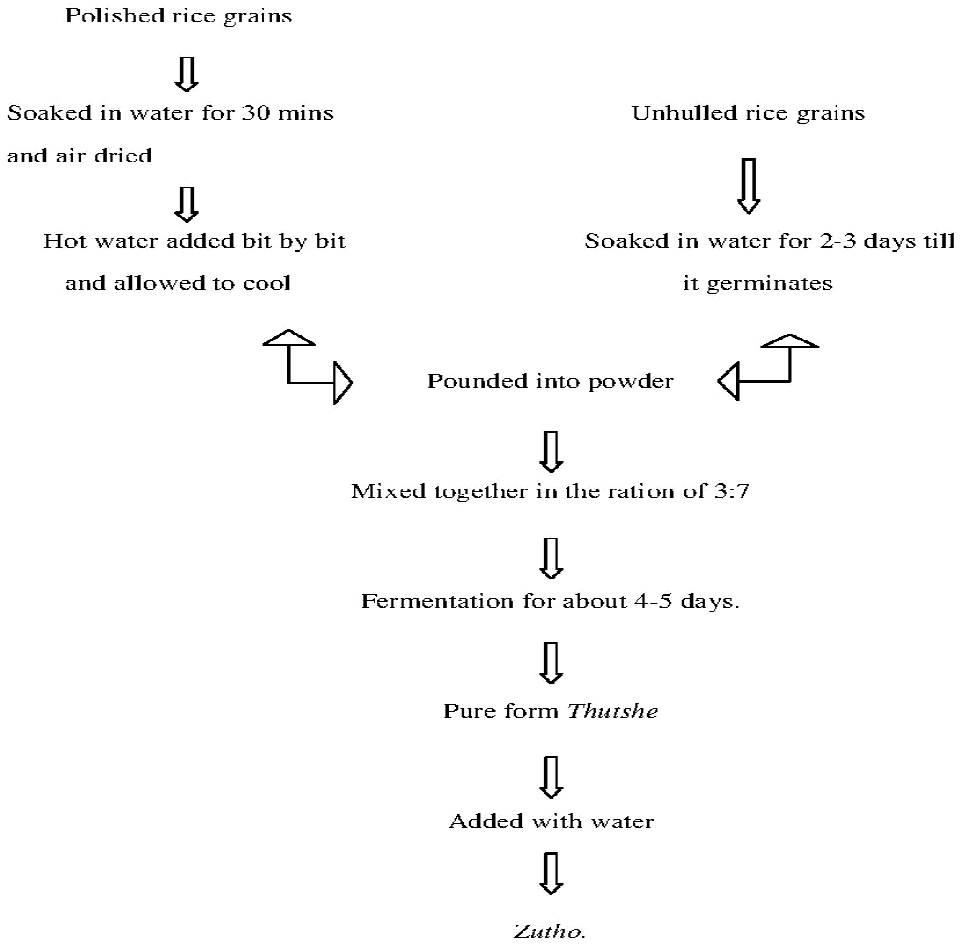


Figure - 2.1

Figure - 2.1: Flow chart of steps in preparation of *Zutho*

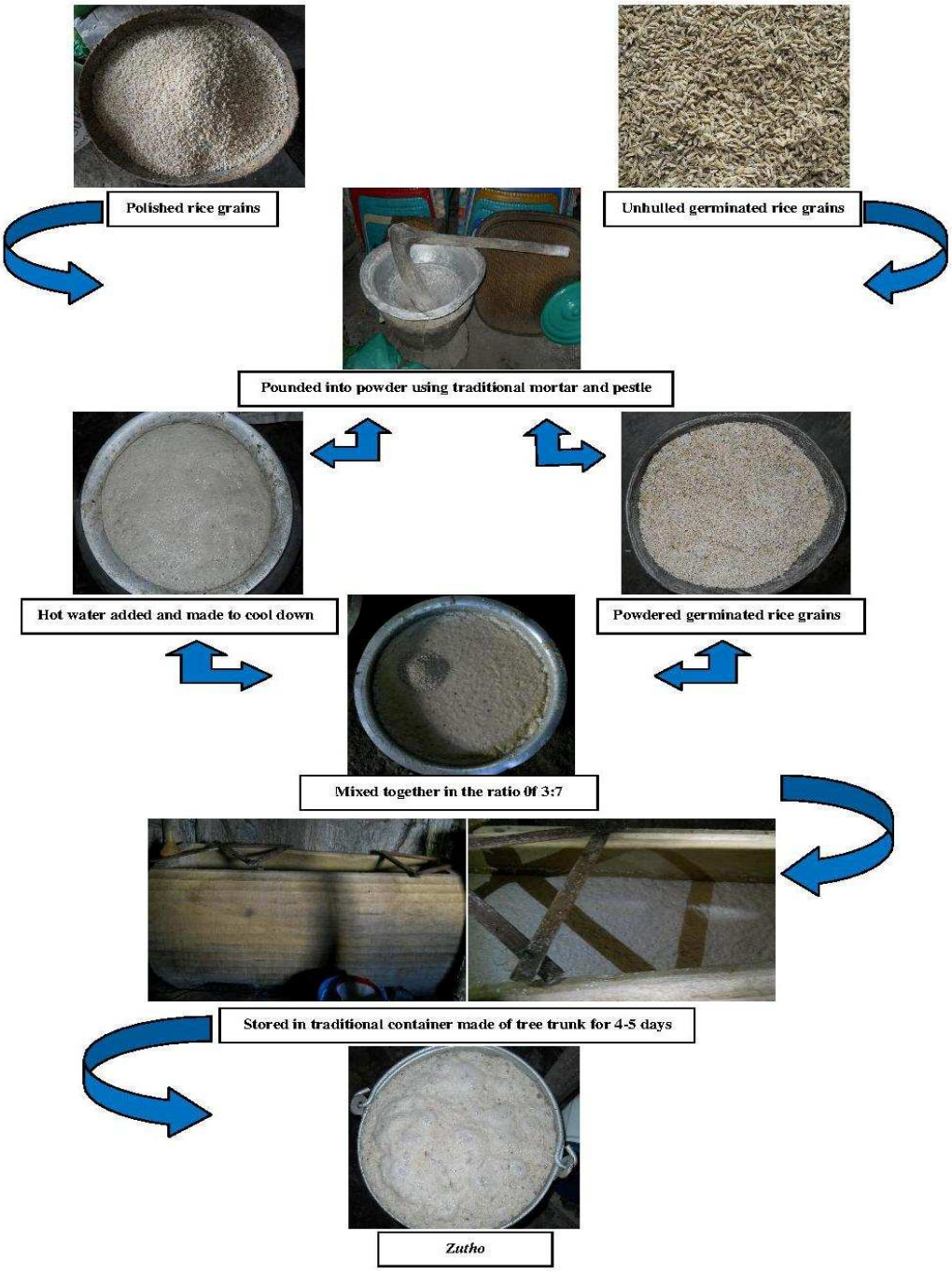


Figure - 2.2

Figure - 2.2: Pictorial steps in preparation of *Zutho*

(ii) *Axone/Akhone*

'*Axone*' is a fermented soybean (*Glycine max* L.) product, named according to the *Sema Naga* dialect. Soybean seeds are first washed and then cooked till it becomes soft. Cooked beans are allowed to cool, and packed in bamboo basket with the base lined with leaves of *Ficus* species which is then covered with the same on top. The bamboo basket is then kept above the fire place to ferment naturally for about 3-4 days. Usually at this point of the step the final product of most of the other fermented soybean product are produced but during *akhuni/axone* preparation it is further made into a paste and then wrapped in banana leaves or *Phrynium pubinerve* leaves and kept above the fire place for about 3-4 days to undergo further fermentation (**Figure 2.3, 2.4**). Most people go for longer fermentation to reduce the strong smell of the fermented product and to increase the shelf life.

Socio-economic importance: *Axone/akhuni* is prepared mostly by the womenfolk in the household level and consumed as a popular condiment by almost all the tribes in Nagaland. It is sold in the local market for ~20 INR per packet and serves as a major source of income for some people. The flow chart of preparation is shown below:

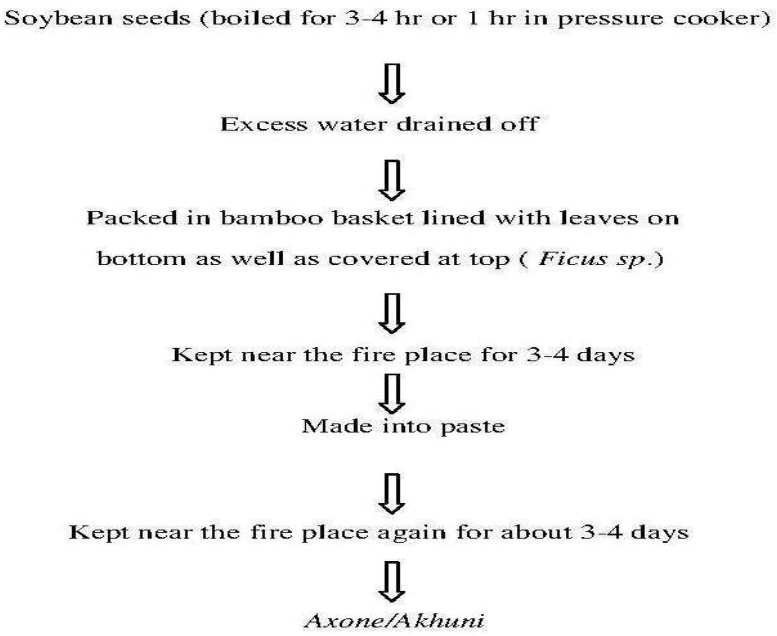


Figure - 2.3

Figure - 2.3: Flow chart of steps of preparation of *Axone*

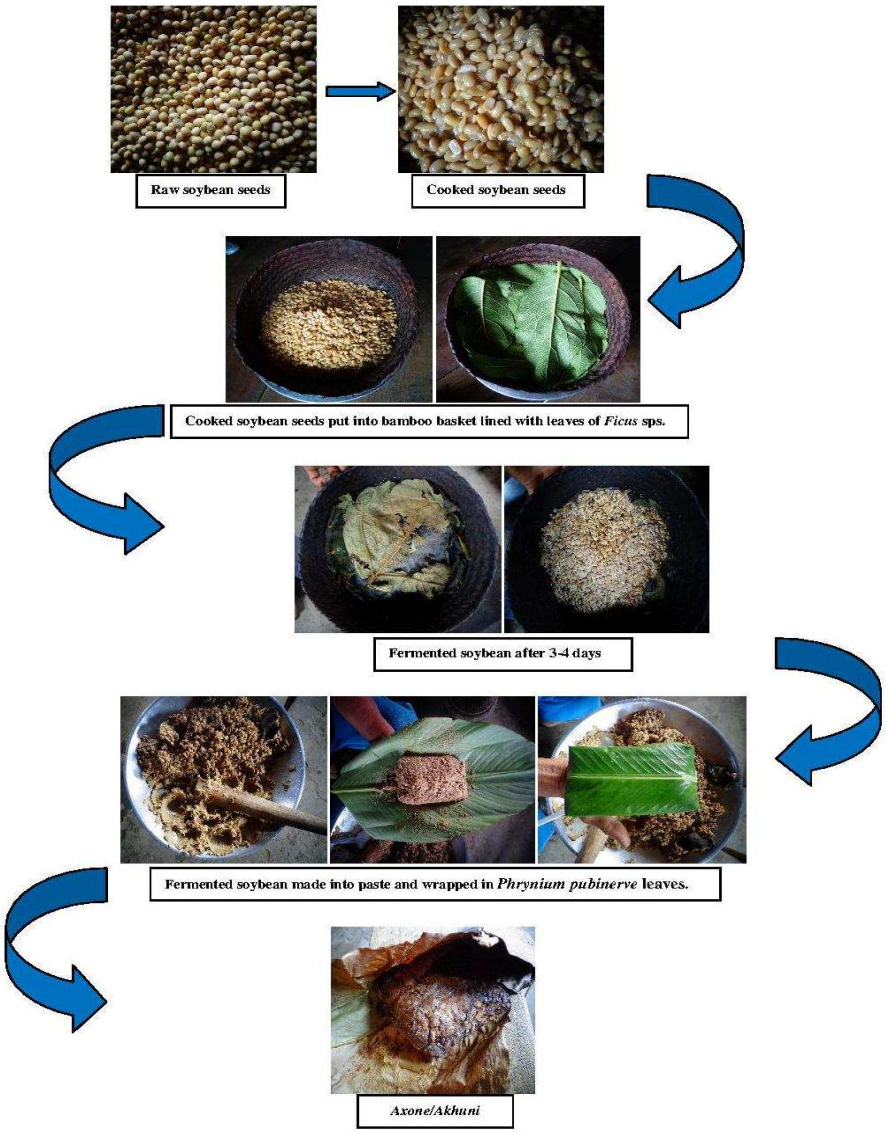


Figure - 2.4

Figure - 2.4: Pictorial steps in preparation of *Axone*

2. Vegetable Based Fermented Food

(i) *Anishi*

'*Anishi*' is a fermented cake made from *Colocasia* leaves (*Colocasia esculenta* L.). It is exclusively prepared by the *Ao Naga* tribe. Its preparation involves the packing of the *Colocasia* leaves in gunny bags or wrapped in banana leaves for about 3-4 days till the leaves becomes yellow. It is then, pounded into pastes which are made into cakes. These cakes are then wrapped in banana leaves and kept under the hot ash near the fire place or exposed to the sunlight till it is completely dried and becomes hard (**Figure 2.5, 2.6**).

Socio-economic importance: *Anishi* is consumed as a popular condiment and can be kept for long period of time. It is produced at a small scale household unit in *Sungratsü* and sold for ~300 INR per kg. The flow chart of preparation is shown below:

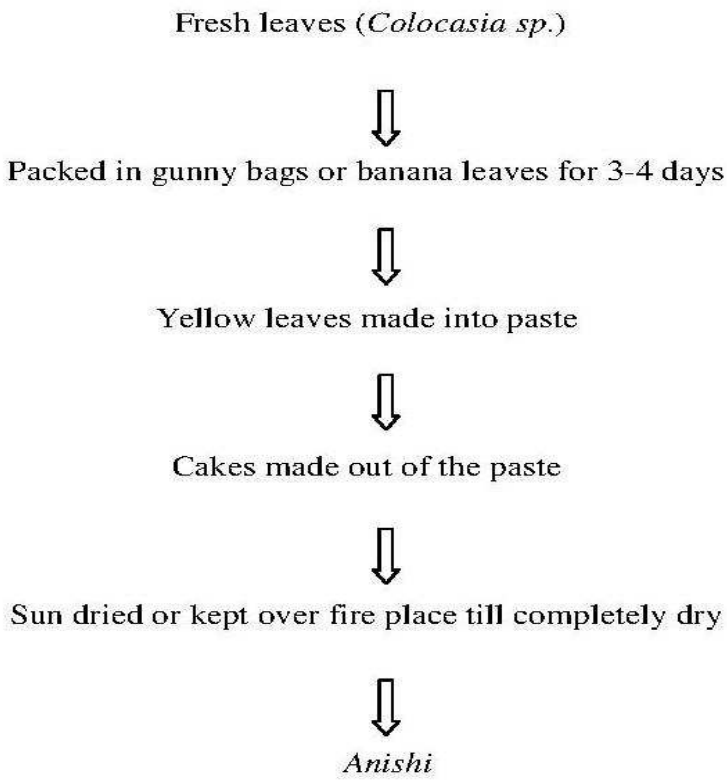


Figure - 2.5

Figure - 2.5: Flow chart of steps in preparation of *Anishi*

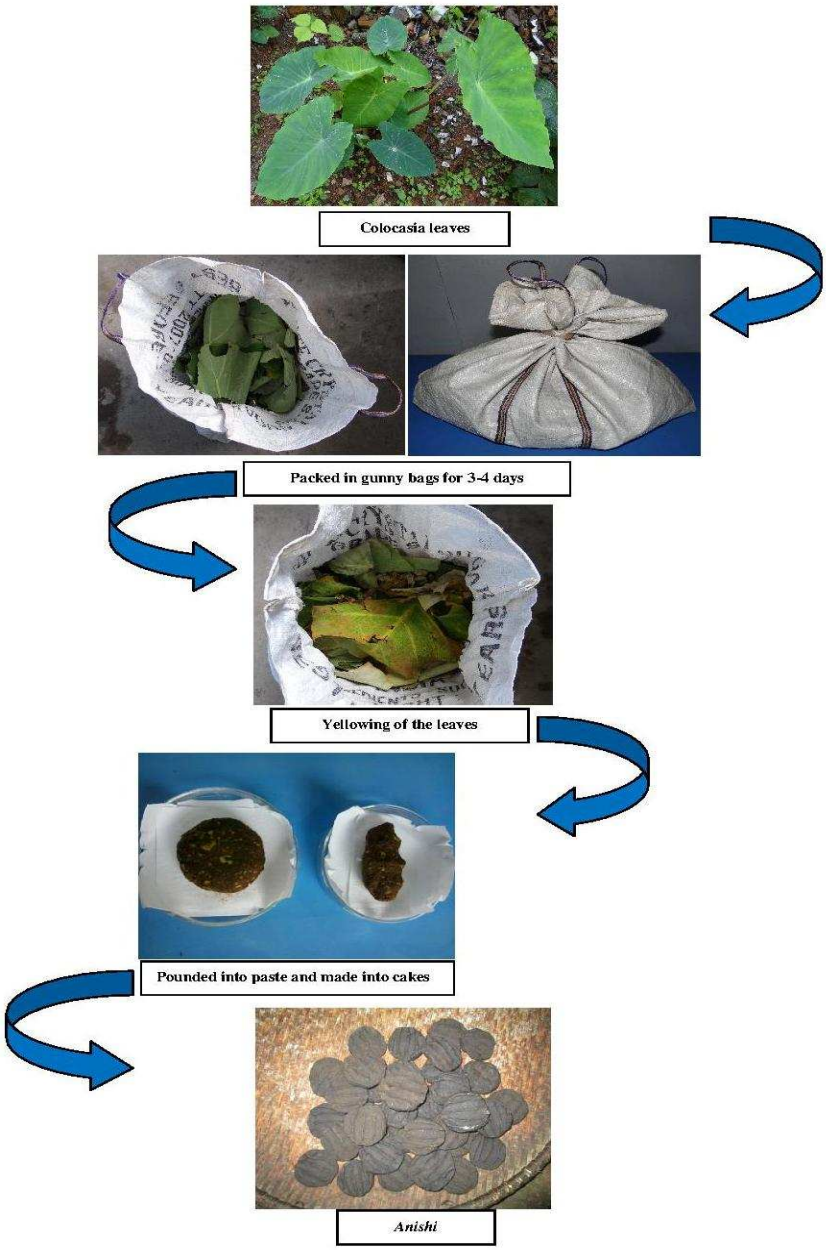


Figure - 2.6

Figure - 2.6: Pictorial steps in preparation of *Anishi*

(ii) *Hungrii*

'Hungrii' is a fermented product prepared from *Brassica* leaves (*Brassica juncea* L.) commonly prepared by the *Rengma* Naga tribe. Pit-fermentation method is followed during its preparation; where a pit of about 2-3 feet is dug on the ground and lined with banana leaves. The leaves are sun dried and allowed to wither. It is then pressed tightly into the pit and covered or plastered with mud on top. It is allowed to ferment naturally for about 15-18 days, after which they are again sun dried to get the final product (**Figure 2.7, 2.8**).

Socio-economic importance: *Hungrii* can be kept for 2-3 years and are consumed as a condiment. They are prepared in bulk during the peak season when brassica leaves are plenty. They are sold for ~400 INR per kg. The flow chart of preparation is given below:

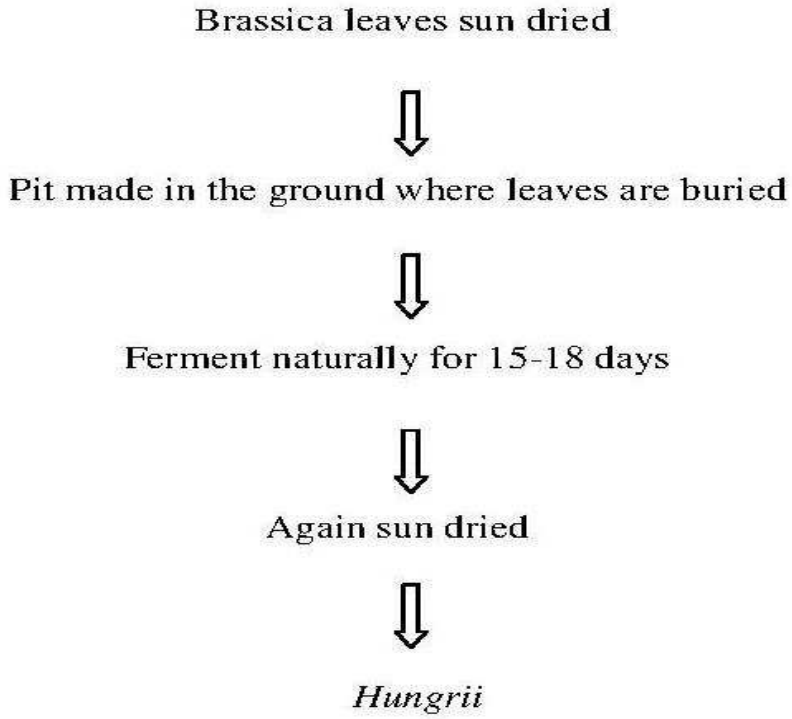


Figure - 2.7

Figure - 2.7: Flow chart of steps in preparation of *Hungrii*

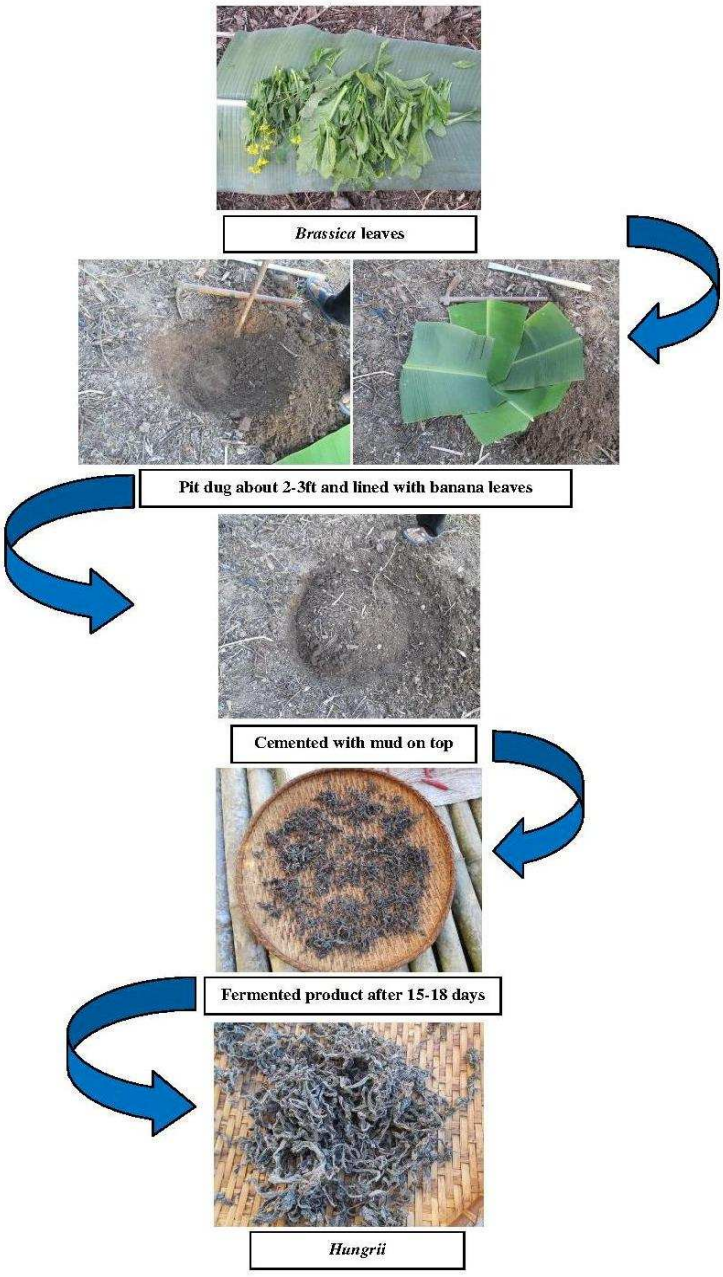


Figure - 2.8

Figure - 2.8: Pictorial steps in preparation of *Hungrii*

iii) *Tsutuocie*

'*Tsutuocie*' is a cucumber based fermented product popularly prepared by the *Angami* Naga tribe. For the preparation, matured and ripened cucumber is first peeled and the seeds are removed. They are then cut into pieces and put into jars or earthen pots along with water and allowed to ferment for about 3-4 months (**Figure 2.9, 2.10**). It is used as a condiment during cooking. *Tsutuocie* can be kept for over 5-6 years.

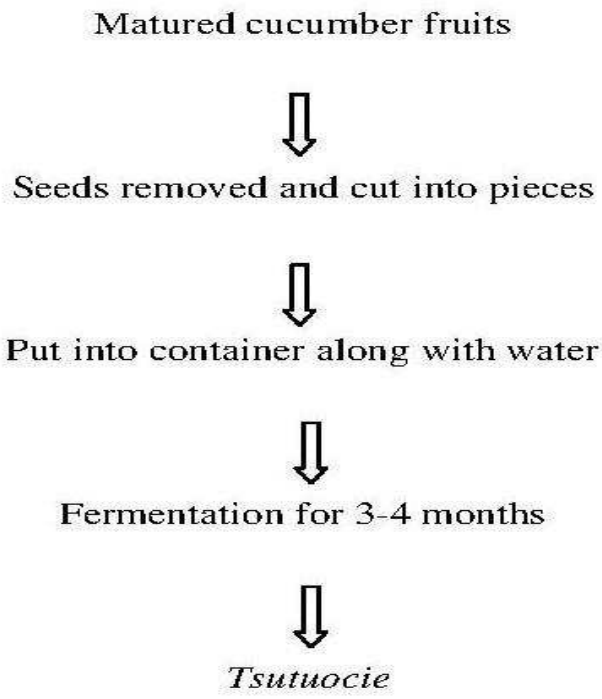


Figure - 2.9

Figure - 2.9: Flow chart for preparation of *Tsutuocie*

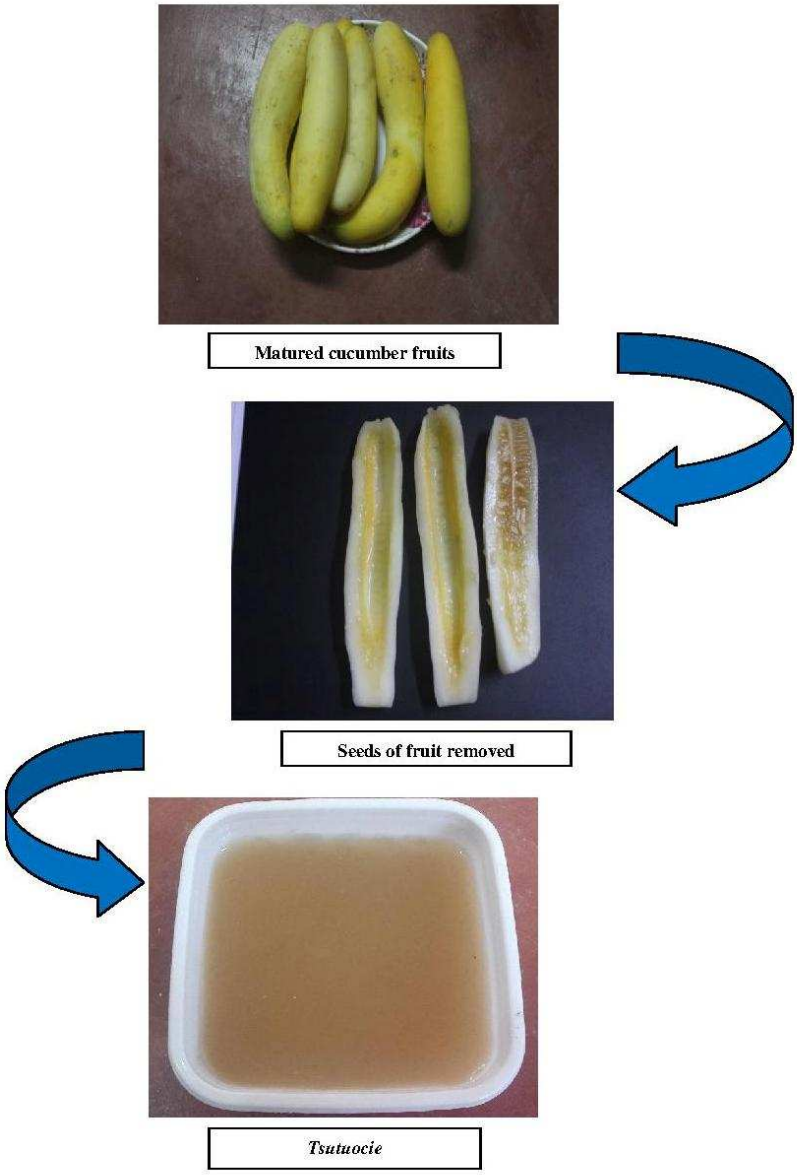


Figure - 2.10

Figure - 2.10: Pictorial steps in preparation of *Tsutuocie*

3. Bamboo Shoot Based Fermented Food

(i) *Rhujuk/Bastanga*

Bastanga is made from succulent bamboo shoots (*Bambusa tulda* Roxb., *Dendrocalamus hamiltonii* Nees et Arn. ex Munro). It is prepared mostly by the *Lotha Naga* tribe, named *Rhujuk* in Lotha dialect .Young shoots are taken and their sheaths are removed till only the soft white part of the shoot remains. The shoot is then pounded slightly and pressed tightly into bamboo baskets lined with banana leaves. A hole is made in the middle so as to let the juice drain out. The preparation is kept in that manner for about 2-3 weeks till the bamboo shoot is completely drained out of its juice. The fermented bamboo shoot is then dried. Different grades of dried bamboo shoots are obtained depending on the way they are cut (**Figure 2.11, 2.12**).

Socio-economic importance: *Rhujuk/bastanga* is consumed as a popular condiment. The fermented bamboo shoot juice can also be stored for years. The thick paste of bamboo shoots is sold for 100-200 INR per container and the dried bamboo shoots are sold for 300-400 INR per kg.

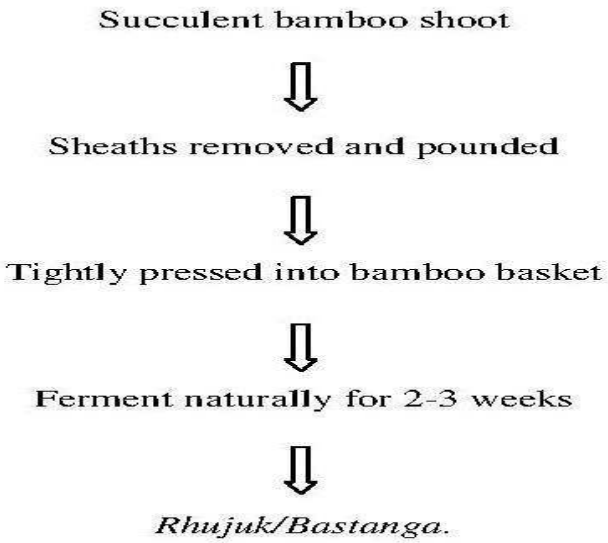


Figure - 2.11

Figure - 2.11: Flow chart of preparation of *Rhujuk/Bastanga*

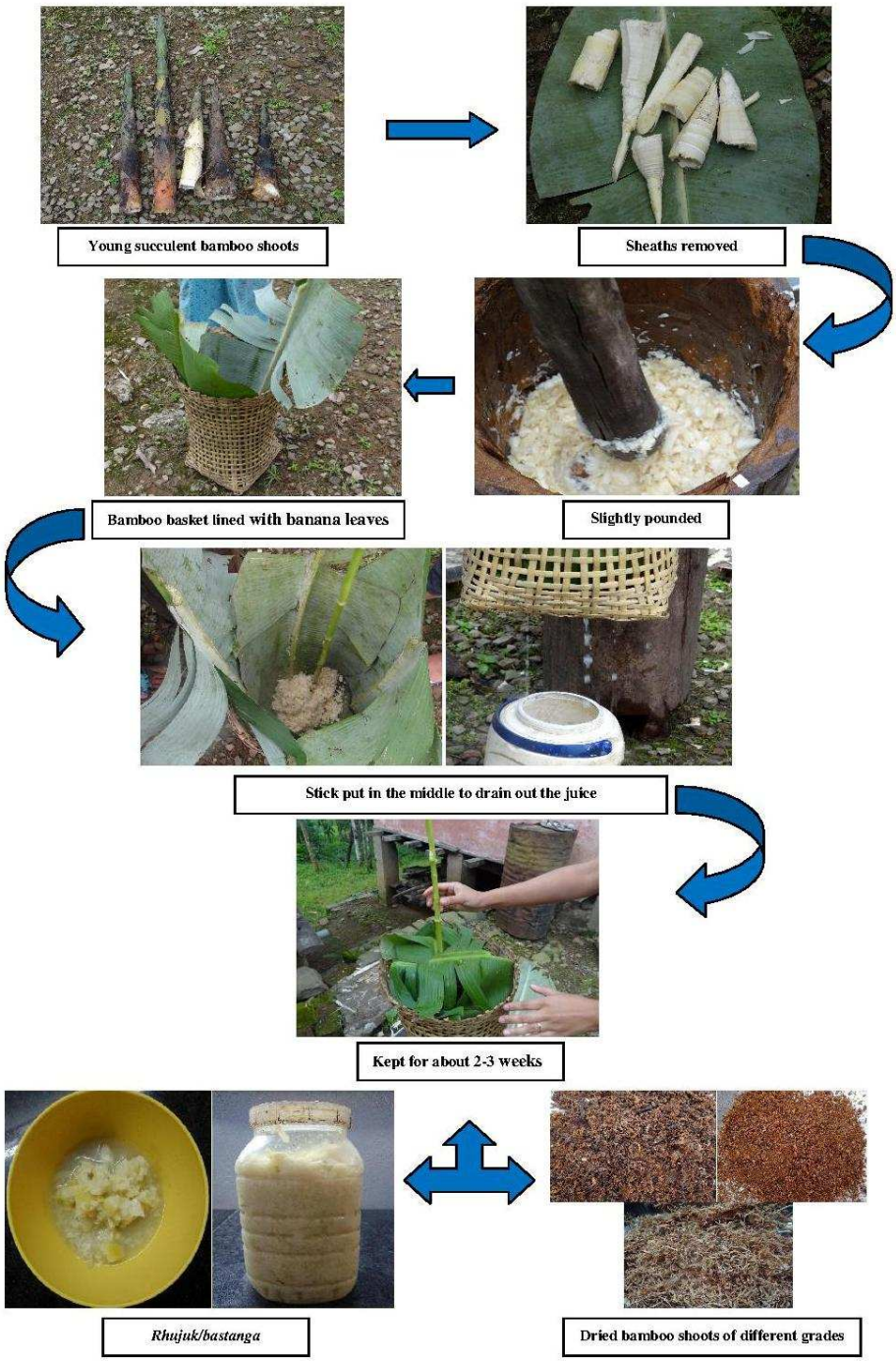


Figure - 2.12

Figure 2.12: Pictorial steps in preparation of *Rhujuk/Bastanga*

4. Meat Based Fermented Food

(i) *Jangpangngatsu*

'*Japangangngatsu'* is a fermented food product made from crab (*Scylla* species), named according to the *Ao Naga* dialect. Crabs are first washed thoroughly and shredded into pieces leaving the hard coverings. The shredded crabs are then made into paste. Black '*til'* (*Sesamum orientale* L.) are slightly simmered and grounded into powder. The mixture of the two are then wrapped in banana leaves or *Phrynium pubinerve* leaf or put into a pot and kept near the fire place for about 3-4 days for the fermentation to be complete (**Figure 2.13, 2.14**).

Socio-economic importance: *Jangpangngatsu* is used for preparation of chutneys. It is sold for 150-200 INR per box. The flow chart of preparation is given below:

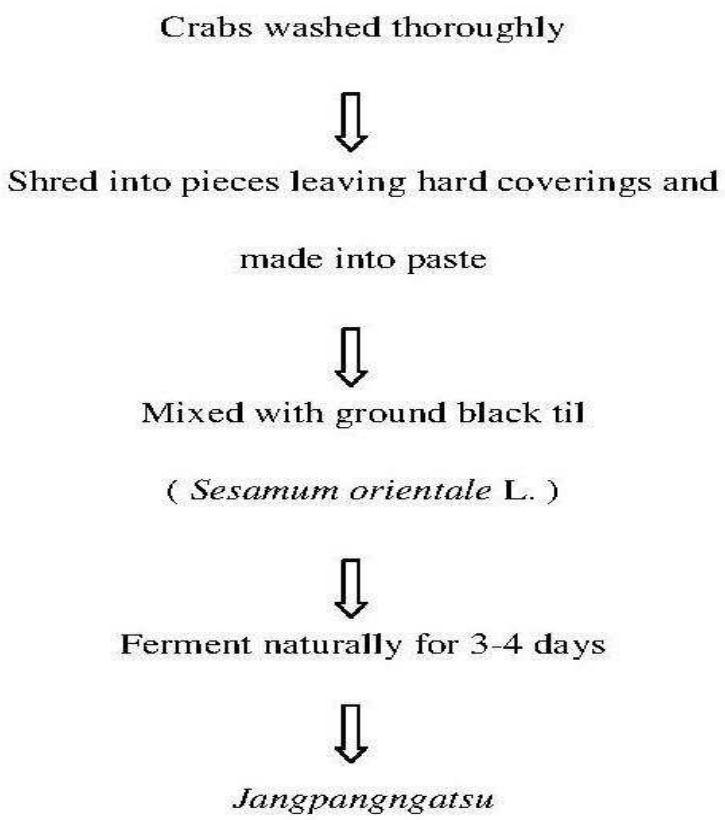


Figure - 2.13

Figure 2.13: Flow chart of preparation of *Jangpangngatsu*

Figure - 2.14

Figure 2.14: Pictorial steps in preparation of *Jangpangngatsu*

(ii) *Jang Kap*

'*Jang kap'* is made from buffalo skin, named according to the *Ao Naga* dialect. The skin is separated from the flesh completely and stacked in a tin or pot with tight covering. It is kept for about 1 wk to allow the fermentation process. After the hairs are completely scrapped off it is either dried in the sun or kept above the fire place. The product is usually pressure cooked and consume as it becomes hard after it is dried. It is a lesser known fermented product consumed by only a few populations. The flow chart of preparation is shown in **Figure 2.15**.

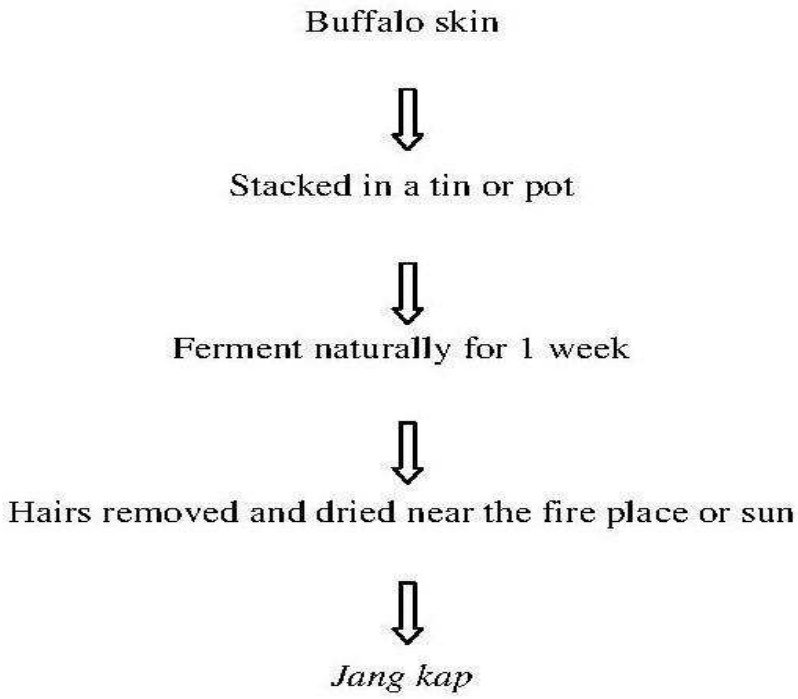


Figure - 2.15

Figure 2.15: Flow chart of preparation of *Jang kap*

iii) Fermented Pork Fat

Pork fat is fermented and taken as a condiment during preparation of vegetables and curries by almost all the *Naga* tribes. Pork fat is cut into small pieces and boiled. It is then put into bamboo containers and the mouth of which is sealed with banana leaves. The fermentation process gets completed in about 1 wk time. It is also a lesser known fermented food product in Nagaland. The preparation steps are shown below (**Figure 2.16**).

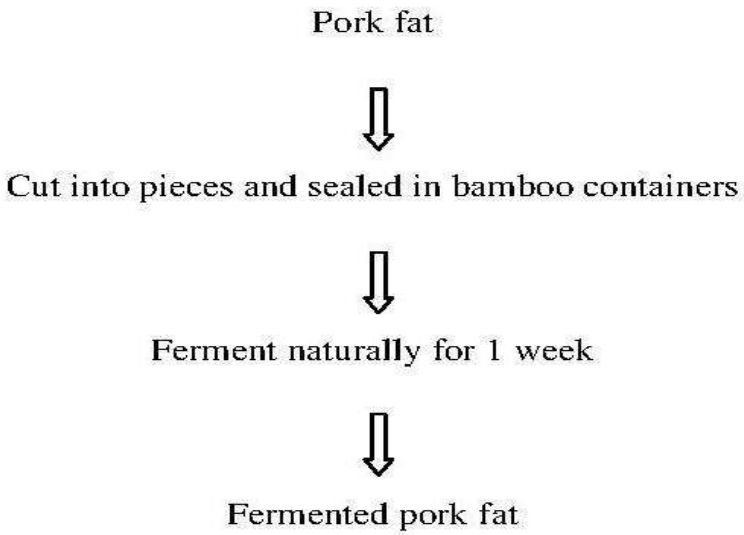


Figure - 2.16

Figure 2.16: Flow chart for the preparation of fermented pork fat

5. Fruit Based Fermented Beverage

The different Naga tribes usually prepare various kinds of fruit beverages from fruits like Naga apple (*Docyna indica*), passion fruit (*Passiflora edulis*), plum (*Prunus* sps.) and gooseberry (*Phyllanthus emblica*). During the preparation of fruit beverages using Naga apple and gooseberry, fruits are collected. Skin and seeds are removed, boiled in water and allowed to cool slightly. Sugar is then added and it is kept for 1-2 wk for fermentation. The pulp of passion fruit and plum are directly soaked in sugar syrup (**Figure 2.17**).

Socio-economic importance: The fermented products are taken as a beverage. It is sold for 150-200 INR per litter in the local market. The preparation step of different beverages is shown below (Figure 2.17):

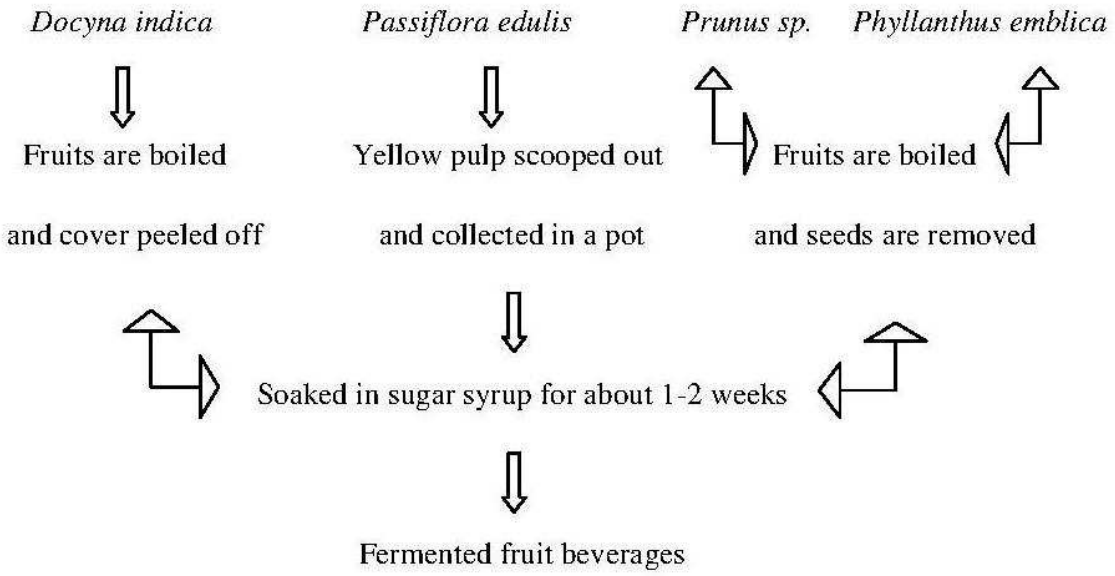


Figure - 2.17

Figure 2.17: Flow chart of preparation of different fruit beverages

Results from the documentation study revealed that, there are varieties of fermented food and beverages that are produced and consumed on a daily basis by the people in Nagaland. Preparation of these fermented foods, in a way helps the people to be self sufficient and gives food security to the local people. These fermented foods and beverages are produced using low cost and locally produced raw materials. The preparation techniques carried out during its preparation are still adaptation from generations on and needs improvisation so as to produce quality products free from contaminations and which are commercially acceptable.

Discussion

Diversity of ethnic fermented foods is related to diversity of ethnicity with unparallel food culture of each community. The ethnic fermented foods provide food security and sustain the livelihood of the people (Tamang et al., 2012). The ethnic people use their traditional knowledge of preserving various raw materials to produce products which are cost effective and are deeply rooted to their cultural heritage. This knowledge is being passed down from generation to generation. Despite rapid urbanization traditional fermented foods in Nagaland are still prepared at the household level and have not been commercialized due to lack of standard quality product. However, some of these fermented products has found their way into the local markets and are produced in small scale production centres. It has helped in the income generation of the local people. The use of their native knowledge of preservation by the local people of various raw materials without using starter culture and chemicals was documented, both as low-cost ethnic foods, and for socio-cultural reasons.

Traditional or indigenous fermented foods are those popular food products that since early history have formed an integral part of the diet and that can be prepared in the household or in cottage industry using relatively simple techniques and equipment (Aidoo

et al., 2006). Documentation of the various fermented food products were done with an aim to preserve the traditional knowledge as well as to bring these products to the limelight so that during the course of time it can be commercialized. There is a need to educate the people on the need to consume fermented foods for security and safety (Hasan et al., 2014). But, one of the major issue pertaining these traditional fermented products are due to lack of proper hygiene, management technique and lack of awareness of its nutritional attributes, these ethnic fermented foods are mostly labelled as unhealthy (Achi, 2005). Several fermented foods and beverages are produced and consumed popularly in almost all the parts of the world. Methods for the production of these fermented food products differ from place to place or from tribe to tribe, as all of them follow their own indigenous protocols employing different starter cultures, although most of them use similar substrate for fermentation.

Cereals and legumes are considered to be the staple foods of the Indian population and are one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fibre for people all over the world (Blandino et al., 2003). However, cereals and legumes contain indigestible oligosaccharides which cause diarrhoea and digestion problems and also anti-nutritional factors like phytic acid and tannins (Nyanzi and Jooste, 2012). Several processes are thus employed to improve the nutritional properties of cereals and legumes and one of the important forms of consumption is the fermented one (Chavan et al., 1989). The first fermentation known to mankind was alcoholic fermentation. It is believed that the discovery of food fermentation was purely by chance and the reports about the origin of fermented foods are lacking. Anthropologists have suggested that it was the production of alcohol which motivated primitive people to settle down and start agriculture for survival (Battcock and Azam-Ali, 2001). Fermented alcoholic beverage *zutho* is prepared from cereal which is similar to other cereal- based

alcoholic beverages like *bhaati jaanr* (Sikkim); *atingba* (Manipur); *apong* (Arunachal Pradesh); *judima* (Assam); *sake* (Japan); *bouza* (Egypt); *jiu* (China) (Tamang and Tamang, 2009; Tamang et al., 2012; Jeyaram et al., 2009; Tamang, 2010; McGovern et al., 2004) . Fermented beverages are reported to have high protein content, vitamin, calcium and are antibacterial (Enujiugha and Badejo, 2017).

Soybean (*Glycine max* L. Merr.) belonging to the *Leguminosae* family is one of the most widely grown crops in the world. The plant has high nutritional value and considered as a good source of protein for humans (Gibbs et al., 2004; Samruan et al., 2012). Traditional fermented soy bean products have been consumed for thousands of years in Asian countries. Fermentation of soybean is one of the techniques resulting in novel foods with unique features (Shin and Jeong, 2015; Tamang, 2015). Soybean in Nagaland is cultivated in kitchen gardens for personal consumption but in parts cultivated on commercial scale and popularly known as '*Naga*' dal. In the present study, *axone/akhuni* is similar to other soybean based fermented products like *kinema* (Sikkim); *hawaijar* (Manipur); *tungrymbai* (Meghalaya); *thou nao* (Thailand); *jang* (Korea); *natto* (Japan) (Sarkar et al., 1994; Jeyaram et al., 2009; Agrahar-Murungkar and Subbulakshmi, 2006; Chukeatirote, 2015; Shin and Jeong, 2015; Hosoi and Kiuchi, 2003). Fermented soybean products are reported to have high protein content as well as significant amount of anti-nutritional factors. Concentration of phenolic compounds also increases, thereby, increasing the antioxidant activity (Mukherjee et al., 2015).

Fermentation of vegetable products applied as a preservation method for the production of finished and half-finished products is considered as an important technology and is further investigated because of the growing amount of raw materials processed in the food industry (Montet et al., 2006). Despite the large diversity in fermented vegetables produced around the world, the type of fermented vegetable used

often depends on the season and geographical area and also on the processing technology used for its preparation (Nguyen et al., 2013). In, Nagaland the most commonly used raw material for vegetable fermentation is mustard leaf, taro leaf and cucumber. These vegetables are collected during the peak of its growing season and preserved by traditional fermentation methods, having longer shelf life. Mustard is the third important oilseed crop in the world. Mustard is cultivated in mostly under temperate climates. It is also grown in certain tropical and subtropical regions as a cold weather crop (Shekhawat et al., 2012). *Hungrii* is similar to other fermented mustard leaf products like *gundruk* and *goyang* (Sikkim); *inziang-sang/inziang-dui* (Manipur); *kimchi* (Korea); *dua cai* be (Vietnam) (Tamang and Tamang, 2009; Wacher et al, 2010). Taro is largely consumed as one of the prominent components of food items and serves as staple source of diet for people around the world and it is the fourteenth most consumed vegetable worldwide (Rao et al., 2010; Buragohain et al., 2013). Most taro varieties contain an irritating or acrid agent and cannot be eaten fresh, so the tubers and leaves are either cooked or fermented traditionally before they are consumed (Chhay et al., 2007). The entire taro plant serves as a source of food, *anishi* is prepared from its leaves but fermented taro corms such as *poi,* and *sapal* are also popular fermented product in Hawaii and Papua New Guinea (Muller et al., 2005; Gubag et al., 1996). Cucumber is one of the oldest and most popular fermented products in the world (Lee, 2001). Though cucumber technically is a fruit, cucumbers are widely considered vegetables. Cucumbers are the fourth most widely cultivated vegetable crop in the world. Cucumber based fermented product *tsutuocie* was found to be similar with other fermented products like *khalpi* (Sikkim); *paocai* (China); *jiang-gua* (Taiwan); *Oiji* (Korea) (Tamang and Tamang, 2009; Di Cagno et al., 2013; Chen et al., 2012; Park and Park, 1998). Fermented vegetable products are reported to contain significant levels of micronutrients, as well as high concentration of

bioactive compounds such as carotenoids, flavonoids, phenol etc contributing greatly to the consumers health (Oguntoyinbo et al., 2016).

Bamboo shoots are the tender young edible shoots which are widely distributed in wild and in mountains from temperate zone of Japan to tropical zone of India (Basumatary et al., 2017). As many as 78 bamboo species (both indigenous and exotic) belonging to 19 genera are being reported from the North-East region of India. Fermented bamboo shoot products and pickles fetch higher income due to the ability of their long-term preservation with higher market price and their consumption throughout the year (Bhatt et al., 2003). Fermented bamboo shoots form a popular delicacy in the traditional cuisines of various tribes and communities of Northeast, India (Choudhury et al., 2012). Fermented product *rhujuk/bastanga* is similar to other bamboo shoot based fermented products like *mesu* (Sikkim); *soibum* and *soidon* (Manipur); *hirring* (Arunachal Pradesh); *lungsiej* (Meghalaya) (Tamang, 2009; Tamang and Tamang, 2009; Agrahar-Murungkar and Subbulakshmi, 2006). Bamboo shoot products are reported to have high level of phytosterols, playing a key role in lowering blood cholesterol and high levels of cellulosic content; high levels of antioxidant activity, minerals and high protein (Waikhom et al., 2015).

Fermented fish products are used to describe the products of freshwater and marine finfish, shellfish and crustaceans that are processed with salt to cause fermentation and thereby to prevent putrefaction (Ishige, 1993). However, *jangpangngatsu* is fermented without added salt but with black '*til*' paste. Spices are often used, acting their role in providing desirable flavours in the fermented products; they facilitate the safe fermentation by inhibiting undesirable or pathogenic microorganisms. They can also have an important role in prolonging shelf-life of fermented meats through their antioxidant

activity (Al-Jalay et al., 1987). Fermented crab product *jangpangngatsu* is similar to *ogiri-nsiko* (Nigeria) (Achi et al., 2007).

Meat is a nutritious, protein-rich food which is highly perishable, with short shelf-life unless preservation methods are used (Campbell-Platt, 1995). Meat is highly nutritious but, in its fresh state, it is perishable and can be an agent for the transmission of a range of infections and intoxications (Adams, 2010). Eating of animal flesh both wild and domesticated is a dietary culture of many ethnic people living in Northeast, India. They usually prefer pork, boar, beef, deer, etc (Tamang, 2009). Meat fermentation is a low energy process which brings various changes in the meat products (Singh et al., 2012). Fermented meat products like pork fat and *jang kap* are similar to other products like *yak chilu, lang chilu* and *luk chilu* (Bhutia, Tibetan); *mogong-grain* (Assam); *sukula* (Nepal) (Rai et al., 2009; Tamang, 2009). Fermented meat products contain bioactive peptides which have antihypertensive, antioxidant, anti-cancer and antimicrobial properties (Zhang et al., 2010).

Fermented fruit beverages are non alcoholic beverages produced from a variety of fruits. Fermented fruits beverages produced around the world are wine prepared from grapes, banana, jackfruit (Battcock and Azam-Ali, 1988); *makumbi* of Zimbabwe prepared from fermented fruit mashes (Gadaga et al., 1999); fruit beverages *tepache* and *colonche* of Mexico prepared from pineapple, apple, orange and pear fruits (Romero-Luna et al., 2017).

Many valuable bio-products around the world are the result of fermentation, either occurring naturally or through addition of starter cultures. Presently, modern technologies and large scale production exploit defined species of starter cultures to ensure consistency and quality in the final product (Mishra et al., 2017). A starter culture may be defined as a preparation containing large numbers of desired microorganisms, used for accelerating

the fermentation process (Holzapfel, 1997). The Food and Agriculture Organisation of the United Nations has stated that value added through marketing and processing raw products can be much greater than the value of primary production (Battcock and Azam-Ali, 1998). Examples are soy sauce, which is a traditional soybean based fermented product that have been innovated by adding enzymes and acids and have become a more safe and consistent product. It is now internationally known and consumed (Luh, 1995; Chen et al., 2012). Yogurt is another product which is innovated by pasteurizing and homogenizing the milk to improve the consistency of the yogurt. Stabilizing compounds such as starch, pectins etc and flavouring agents are added providing variety to the consumers (Soukoulis et al., 2007). Sauerkrauts, a traditional vegetable based fermented product is produced in large scale in industries. Use of pure starter cultures and controlled environment i.e., ambient temperature and salt concentration affects the quality of the final product (Vieira and Ernest, 1996).

Summary and Conclusion

In this chapter preparation of some of the popular fermented food products of Nagaland are described. These descriptions are based on the ethnic preparations which are being followed from last several generations without any scientific look. Latest research or improvements made in any field need to be able to sustain the transfer of technologies. So to improve any technology it is essential that current technology for production of indigenous fermentation is documented. Nagaland inhabited by diverse tribal communities produces varieties of fermented foods and beverages. Fermented foods and beverages in Nagaland are produced mainly for food preservation and to meet the growing needs of the people. Some of these fermented products are not only produced for one's own consumption but are also produced to be marketed in the local market. These fermented food products have their own unique way of preparation and it is

essential that this knowledge be preserved. Most of these fermented food and beverages are associated with a particular tribe and in a way give a cultural identification value for communities through its food. Thus, fermented foods play a very important role in the culture, religion and economic status of the *Naga* people.

Indigenous methods of food preparation should be upgraded to faster, better, and cleaner modern methods without prejudice or nostalgia of its traditional value, as these can lead to better nutrition and health. Thus, there is a need to intensively study and develop the traditional fermented products for better quality so as to benefit and improve the health as well as to commercialize these products at a larger scale. Increased documentation and efforts for sharing this information will allow taking advantage of this vast knowledge. Indigenous foods will remain important as long as they are adapted to a changing environment characterized by dynamic tastes and preferences. Biotechnological intervention to assess the nutritional value and to identify the microorganisms present in these traditional fermented foods, are the need of the hour. Thus, the following chapters deal with the nutritional assessment and the microbiological studies of five popularly consumed fermented products of Nagaland, viz., *axone/akhuni, anishi, hungrii, rhujuk/bastanga* and *tsutuocie.*

Chapter - 3

Microbiology and Molecular Identification of Dominant Microorganisms in the Fermented Food Products

---

Microorganisms had been unknowingly subjected to undergo natural fermentation from ancient history. Without knowledge of microbes, our ancestors recognized, over time, the palatability, preservative, analgesic, and mentally stimulating or sedating qualities of fermented foods and beverages (Steinkraus, 2002). Steinkraus (1997) defined fermented foods as food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids respectively to non-toxic products with flavours, aromas and textures pleasant and attractive to the human consumer. If the products of enzyme activities have unpleasant odours or undesirable smell, have unattractive flavours

or the products are toxic or disease producing, then the foods are described as spoiled and not fermented. A wide spectrum of microorganisms is involved during fermentation processes but only a few types usually determine quality of the end product (Abegaz, 2007).

Microorganisms may be present as natural indigenous microbiota in uncooked plant or animal substrates, utensils, containers, earthen pots, or environments or as added starter cultures containing functional microorganisms (Hesseltine, 1983; Tamang et al., 2016). Fermentation may magnify the known benefits of a wide variety of foods and herbs, influencing the bioavailability and activity of the chemical constituents. In addition, as our knowledge of the human micro biome increases (the intestinal microbiota in particular), it is becoming increasingly clear that there are untold connections between the ways in which microbes act upon dietary items pre-consumption, and in turn, the ways in which these fermented dietary items influence our own microbiota (Selhub et al., 2014). Microorganisms determine the characteristics of fermented food, where the raw materials are converted by microorganisms (bacteria, yeasts and moulds) to products that have acceptable food qualities as well as health benefits that go beyond simple nutrition (Steinkraus, 2002; Giraffa, 2004; Vogel et al., 2011; Tamang et al., 2016). Some microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant flora during the course of the fermentation. The common fermenting bacteria are species of *Lactobacillus*, *Lactococcus Leuconostoc*, *Enterococcus, Streptococcus*, *Pediococcus*, *Micrococcus* and *Bacillus.* The fungi genera *Aspergillus*, *Amylomyces, Actinomucor, Paecilomyces, Monascus, Mucor, Neurospora, Cladosporium*, *Fusarium*, *Penicillium* and *Rhizopus* are the most frequently found in certain products. The common fermenting yeasts are species of *Candida, Debaryomyces, Geoirichum, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Saccharomycopsis,*

Torulopsis and *Zygosaccharomyces* (Steinkraus, 1998; Tamang, 1998; Tamang et al., 2016).

Functional properties of microorganisms in fermented foods include probiotics properties, antimicrobial properties, antioxidant, peptide production, fibrinolytic activity, poly-glutamic acid, degradation of antinutritive compounds etc. which may be important criteria for selection of starter culture(s) to be used in the manufacture of functional foods (Tamang et al., 2016). Lactic acid bacteria are a group of gram-positive, non-spore forming, coccus or rod shaped bacteria. They ferment carbohydrates to almost entirely lactic acid (homofermentation) or to a mixture of lactic acid, carbon dioxide and acetic acid and/or ethanol (heterofermentation). Other compounds, such as diacetyl, acetaldehyde and hydrogen peroxide, are also produced. These compounds contribute to the flavour and texture of fermented foods and may also contribute to the inhibition of undesirable microbes (Swain et al., 2014; Nuraida, 2015). Some strains of *Bacillus* species are reported to have antimicrobial activity and also produce several enzymes such as proteinase, amylase, mannase, cellulase and catalase (Ouoba et al., 2007; Tamang, 1998). Yeast are reported to have the function to convert carbohydrates into alcohols and other aroma compounds such as esters, organic acids and carbonyl compounds in the fermentation of foods and beverages (Janssens et al., 1992; Czerucka et al., 2007). During the growth of fungi, the functional properties of foods are formed as follows: protein is hydrolyzed to amino acids and peptide by proteolytic enzymes, oligosaccharides is hydrolyzed to monosaccharides, phytic acid degraded to inorganic phosphates (Handoyo and Morita, 2006; Bourdichon et al., 2012; Chen et al., 2014). Species of lactic acid bacteria like *Bacillus*, amylolytic and alcohol-producing yeasts and filamentous moulds are the major microbiota in the fermented foods and alcoholic beverages of Asia, whereas LAB or a combination of bacteria (LAB, non-LAB , micrococci) - yeast mixtures and

filamentous moulds are more prominent in Africa. Filamentous molds and bacilli are rare in the fermented foods and beverages of Africa, Europe, Australia, and America (Tamang, 1998), although fermented legume products, based on *Bacillus* fermentation, are common in West Africa (Oguntoyinbo et al., 2007).

Several microbiological studies have dealt with the characterization and identification of microorganisms isolated from various fermented foods as a first step in improving the quality of these foods through the development of appropriate starter cultures. Evaluating microbial diversity in fermented food is problematic because it is often difficult to cultivate most of the viable bacteria or to detect stressed cells. This has led to the introduction of molecular identification which is an accurate and reliable identification tool, and is widely used in identification of both culture-dependent and culture-independent microorganisms from fermented foods (Giraffa and Neviani, 2001; Cocolin et al., 2013). PCR-denaturing gradient gel electrophoresis (PCR-DGGE) and PCR-temperature gradient gel electrophoresis (PCR-TGGE) were introduced in the later part of 20^{th} century in environmental microbiology and are now routinely used in many laboratories worldwide as molecular methods to study population composition and dynamics in food-associated microbial communities (Muyzer et al., 1993; Cocolin et al., 2000). Greppi et al. (2013) first reported the combination of both culture-dependent and independent methods to reveal predominant yeast species and biotypes in the traditional fermented maize foods of Benin. In these studies, the 16S rRNA gene is PCR-amplified from a DNA sample, the amplicon is sequenced, the sequence is queried on a database like the NCBI, sequence hits are pooled from the database, and the sequences are used for phylogenetic analysis. The gene databases have facilitated these studies and as a result bacterial diversity surveyors are able to share information eliminating the need for

repeated surveys. Various regions of the 16S rRNA gene can be explored to study variation among bacterial phylogenies (Ntushelo, 2013).

The 16S rRNA gene sequence is a component of the 30S subunit (Smaller subunit of the 70S ribosome) of prokaryotic ribosome consisting of 1550 bp. This sequence has both conserved and variable regions. The conserved region was discovered in the 1960s by Dubnau et al. (1965) in *Bacillus* spp. Universal primers are usually used as complementary to this region. The 16S rRNA gene is the most commonly used part of the DNA for bacterial identification and comparison purposes (Clarridge III, 2004). Some scientists refer to this gene as 16S rDNA; however, the approved name is 16S rRNA. It is the most popular gene sequence used in identifying organisms because it behaves like a molecular chronometer (Stackebrandt et al., 2002; Selvakumaran et al., 2008). This conserved region is found in all bacteria as such it allows for comparisons and differentiation among bacteria (Woese et al., 1985). The 16S rRNA gene is a suitable parameter for bacterial classification because the 16S rRNA gene is universal among bacteria and is conserved but has sufficient variation to distinguish between taxa (Ntushelo, 2013). The objectives of this study were to isolate the microorganisms present in the fermented food products, and to identify them using classical microbiological and DNA-based identification methods. For the latter, sequence analysis of the genes encoding 16S ribosomal RNA was used.

Materials and Methods

Materials

Five samples each of the five different fermented foods viz., *axone/akhuni, anishi, hungrii, rhujuk/bastanga* and *tsutuocie* were taken from household products collected randomly from in and around Nagaland. Samples were kept in a refrigerator at 4°C until processed.

Methods

Estimation of *p*H

Five gram of sample was blended with 10 ml of distilled water in a homogeniser and the *p*H of the slurry was determined directly using a digital *p*H meter.

Microbiological study

Preparation of serial dilutions

Samplings were performed on stored finished fermented products to examine for its microbial groups. Ten grams of sample were taken aseptically and homogenized in sterile physiological saline (peptone, 0.1% w/v; NaCl, 0.85% w/v,) for 1 min. Then serial dilutions were prepared by transferring one ml from first dilution (10^{-1}) to 9 ml peptone water and serially diluted further up to 10^{-10} dilutions with saline water as described by Harrigan and McCane (1976). Then plate counts were carried out using the following media, temperature and incubation periods to enumerate different microbial group.

Total viable bacteria count

To determine the total bacterial count 0.1 ml of serially diluted 0.1% (w/v) sample was inoculated plate count agar (PCA) **(Appendix-II)** and incubated at 30-32ºC for 48h. Colony forming units (CFU) were counted using a colony counter and the results were presented as cfu ml^{-1}.

Enumeration of coliform bacteria

Appropriate decimal dilutions (0.1 ml) of the homogenate was spread on Nutrient Agar and TSA **(Appendix-II)** and incubated at 37°C for 24 h. Members of Enterobacteriaceae were enumerated using Violet red bile glucose agar and incubated at 30°C for 48 h.

Enumeration of Lactic Acid Bacteria

From appropriate dilutions, 0.1 ml aliquots were spread plated in triplicates on pre-dried surfaces of MRS **(Appendix-II)** agar plates supplemented with 1% (w/v) calcium carbonate. The plates were incubated anaerobically in an Anaerobic Gas-Bag system at 30-32°C for 48h.

Enumeration of *Staphylococci*

Selective enumeration was carried out by spread plates on Baird-Parker agar media **(Appendix-II).** The plates were incubated at 37°C for 48 h.

Yeast and Mold Enumeration

From suitable dilution of sample, 0.1 ml was transferred onto solidified PDA and YMA **(Appendix-II),** supplemented with 12 µg ml^{-1} Streptomycin to inhibit bacterial growth. Plates were then incubated at 27°C for 48 h.

Phenotypic Characterization

Morphologically different colonies were isolated and purified cultures were grown on slants of the same medium and stored at 4°C. Purified isolates were checked for gram stain and for catalase production.

Gram staining

Each isolate was smeared on grease free slide, air-dried and heat fixed by passing each slide over the blue flame of a burning Bunsen burner repeatedly. Each slide was flooded with a crystal violet solution, dried for one min, washed with distilled water and treated with iodine for one min. The slides were decolorized by ethanol (90%, v/v), washed with distilled water and counter stained using safranin for 30 sec. Slides were

washed again, air-dried and observed under oil immersion objective lens (x100) of the light microscope (Gram, 1984) **(Appendix-II).**

Catalase test

Microorganisms produce hydrogen peroxide during respiration and in some instance and extremely toxic superoxide leads to the death of the microorganisms. Unless they are degraded enzymatically, organisms capable of producing catalase or peroxidase rapidly degrade hydrogen peroxide. Pure cultures were scooped out on a slide and 4 drops of 3% hydrogen peroxide was added to each bacterial spot. Presence of bubbling or foaming indicates the breakdown of hydrogen peroxide with release of oxygen. The absence of foaming indicated the absence of catalase activity (Aneja, 2003).

Molecular Identification

DNA isolation

Extraction of genomic DNA was done using CTAB protocol described by Moore et al. (2004) with slight modification **(Appendix-IV).** About 5 ml bacterial broths was centrifuged at 10,000 rpm for 5 min at 4°C followed by suspension in 500µL of TE buffer and thoroughly mixed with 200µL of Lysozyme. The mixture was incubated for 45 min at 37°C water bath. To this 10µL of proteinase K and 50µL of SDS were added and mixed thoroughly and incubated at 37°C until the solution became clear and viscous. Subsequently, 100µL of 5M NaCl was then added and incubated at 65°C for 5 min. It was again incubated at 65°C for 10 min after addition of 100µL CTAB solution. The suspension was extracted with equal volumes of phenol: chloroform: iso-amyl alcohol (25:24:1) and centrifuged at 10,000 rpm for 10 min. The upper phase was transferred and to it equal volumes of chilled isopropanol was added and mixed thoroughly by inverting the tubes. Aqueous phase was recovered by centrifugation at 10,000 rpm for 15 min. Isopropanol was removed and the pellet was washed in 70% ethanol by centrifugation at

10,000 rpm for 15 min. The pellets were then allowed to stand for 5-10 min and then re-suspended in 50µL of TE buffer. The extracted genomic DNA was tested qualitatively on 1% (w/v) agarose gel electrophoresis and quantified using Nanodrop Spectrophotometer.

Polymerase Chain Reaction (PCR)

The PCR was performed in a thermal cycler according to Kumar et al. (2002) with some modification under the following standardized conditions. The 16S rDNA gene sequences were amplified using universal primers 9F and 1492R (Lechner et al., 1998) and 27F and 1492R (Lane, 1991) **(Appendix-V).** About 25µL of PCR mixture **(Appendix-VI)** was amplified in a PCR programmed with the following temperatures: 94°C for 5 min then 35 cycles at 94°C 1 min, 60°C for 1 min and 72°C for 30 sec. The final extension was at 72°C for 5 min and stopped at 4°C.

16S rRNA Sequencing

Amplified products were separated by electrophoresis in 1.2%, w/v agarose gel and were purified using a commercial kit (HiPura PCR Product Purification Kit, Make: HiMedia, India). Sequencing was done at 1st Base, Singapore. To determine the closest known relatives of the partial DNA sequences obtained, searches for homologous nucleic acid sequences was performed using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/). Percent similarity values of the most closely related identities were determined by a comparison with the sequences available in the database using BLAST software (Altschul et al., 1990).

Phylogenetic Study

Reference sequences were downloaded from NCBI and comparison was done by using the multiple sequence alignment program CLUSTALW (Thompson et al., 1997). Since BLAST search results showed the most similar reference sequence with query sequences, detailed species level identifications were conducted by phylogenetic analysis.

The neighbour-joining phylogenetic tree (Saitou and Nei, 1987) was constructed using the CLC Sequence Viewer 7.7 for construction of phylogenetic trees, inferring distances and percent similarity. A bootstrap analysis using 1000 replications was performed to assess the relative stability of branches.

Results

Phenotypic Characterization

Microorganisms like filamentous moulds, yeasts and bacteria constitute the microbiota in indigenous fermented foods, which are present in or on the ingredients, utensils or environment, and are selected through adaptation to the substrate. Identification of the microbes in this study was done using the classical culture-dependent method followed by use of molecular tools to identify the microorganisms. The phenotypic characterization was done with morphological studies i.e., gram staining and colony morphology and the genotypic characterization was done using 16S rRNA universal primers.

From the twenty five collected fermented food samples, 119 isolates were isolated (*axone/akhuni*-30; *anishi*-22; *hungrii*-19; *rhujuk/bastanga*-25; *tsutuocie*-23) and recorded. Out of which thirty nine isolates were selected based on grams staining, catalase test and morphology **(Table 3.1-3.5; Figure 3.1-3.6).** Yeasts and moulds were not detected in any of the samples.

Table 3.1: Grams staining, catalase test and morphological characteristics of bacteria isolated from *axone/akhuni* fermented product

Sl. No.	Isolates	Catalase	Gram staining	Cell shape	Colony morphology	Colony colour
1	BJ-DEBCR-2	+	+	Rod	Irregular, large	White
2	BJ-DEBCR-33	+	+	Rod	Irregular, large	White
3	BJ-DEBCR-3	+	+	Rod	Irregular, large	White
4	BJ-DEBCR-24	+	+	Rod	Irregular, large	White
5	BJ-DEBCR-22	+	+	Rod	Irregular, large	Golden brown
6	BJ-DEBCR-29	+	+	Rod	Irregular, large	Golden brown
7	BJ-DEBCR-1	+	+	Coccus	Circular, pinhead	White
8	BJ-DEBCR-21	+	+	Rod	Irregular, small	White

Table 3.2: Grams staining, catalase test and morphological characteristics of bacteria isolated from *anishi* fermented product

Sl. No.	Isolates	Catalase	Gram staining	Cell shape	Colony morphology	Colony colour
1	BJ-DEBCR-6	+	+	Rod	Irregular, large	White
2	BJ-DEBCR-40	+	+	Rod	Irregular, large	White
3	BJ-DEBCR-4	+	+	Rod	Irregular, large	White
4	BJ-DEBCR-17	+	+	Rod	Irregular, large	White
5	BJ-DEBCR-18	+	+	Rod	Irregular, large	White
6	BJ-DEBCR-28	+	+	Rod	Irregular, large	White
7	BJ-DEBCR-41	+	+	Rod	Irregular, large	White
8	BJ-DEBCR-20	+	+	Rod	Irregular, large	White
9	BJ-DEBCR-16	-	+	Coccus	Round, small	Cream

Table 3.3: Grams staining, catalase test and morphological characteristics of bacteria isolated from *hungrii* fermented product

Sl. No.	Isolates	Catalase	Gram staining	Cell shape	Colony morphology	Colony colour
1	BJ-DEBCR-23	+	+	Rod	Irregular, large	White
2	BJ-DEBCR-11	+	+	Rod	Irregular, large	White
3	BJ-DEBCR-19	+	+	Rod	Irregular, large	White
4	BJ-DEBCR-26	+	+	Rod	Irregular, large	White
5	BJ-DEBCR-36	+	+	Rod	Irregular, large	White
6	BJ-DEBCR-35	+	+	Rod	Irregular, large	White

Table 3.4: Grams staining, catalase test and morphological characteristics of bacteria isolated from *rhujuk/bastanga* fermented product

Sl. No.	Isolates	Catalase	Gram staining	Cell shape	Colony morphology	Colony colour
1	BJ-DEBCR-9	+	+	Rod	Irregular, large	White
2	BJ-DEBCR-14	+	+	Rod	Irregular, large	White
3	BJ-DEBCR-30	+	+	Rod	Irregular, large	White
4	BJ-DEBCR-31	+	+	Rod	Irregular, large	White
5	BJ-DEBCR-37	+	+	Rod	Irregular, large	White
6	BJ-DEBCR-39	+	+	Rod	Irregular, large	White
7	BJ-DEBCR-5	+	+	Rod	Irregular, large	White
8	BJ-DEBCR-38	+	+	Rod	Irregular, large	White
9	BJ-DEBCR-32	+	+	Rod	Irregular, large	White
10	BJ-DEBCR-27	+	+	Coccus	Circular, pinhead	White

Table 3.5: Grams staining, catalase test and morphological characteristics of bacteria isolated from *tsutuocie* fermented product

Sl. No.	Isolates	Catalase	Gram staining	Cell shape	Colony morphology	Colony colour
1	BJ-DEBCR-7	+	+	Rod	Irregular, large	White
2	BJ-DEBCR-8	+	+	Rod	Irregular, large	White
3	BJ-DEBCR-25	+	+	Rod	Irregular, large	White
4	BJ-DEBCR-10	+	+	Rod	Irregular, large	White
5	BJ-DEBCR-12	+	+	Rod	Irregular, large	White
6	BJ-DEBCR-34	+	+	Rod	Irregular, large	White

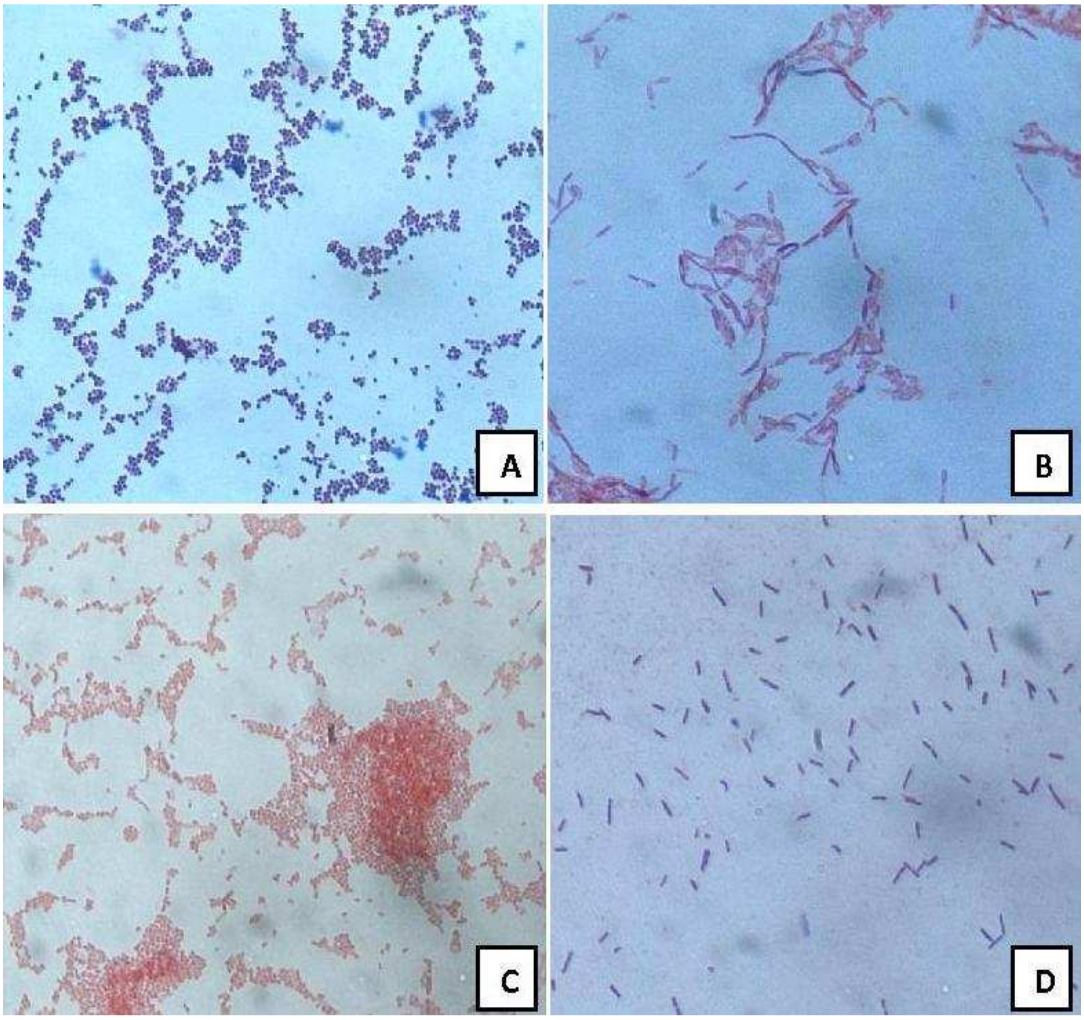


Figure - 3.1

Figure 3.1: Cell morphology of bacteria after gram staining. **A.** Gram positive cocci; **B.** Gram positive rod with endospore; **C**. Gram negative cocci and **D**. Gram positive rod.

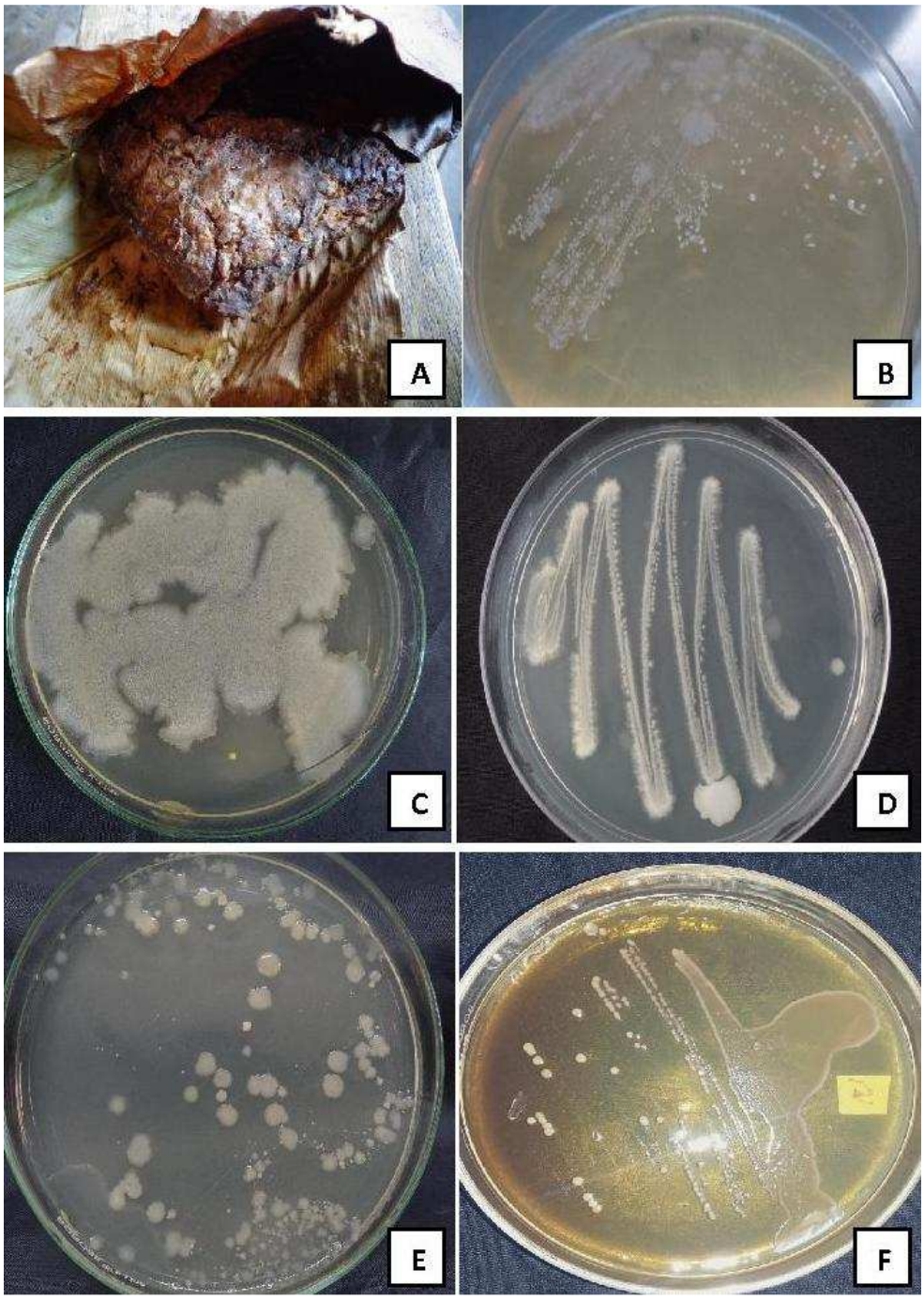


Figure - 3.2

Figure 3.2: Pure cultures of bacterial isolates from *Axone/Akhuni*. **A**. *Axone/akhuni*; **B.** *Staphylococcus epidermis*; **C**. *Bacillus subtilis*; **D**. *Bacillus licheniformis*; **E**. *Alcaligenes faecalis* and **F**. *Bacillus cereus*

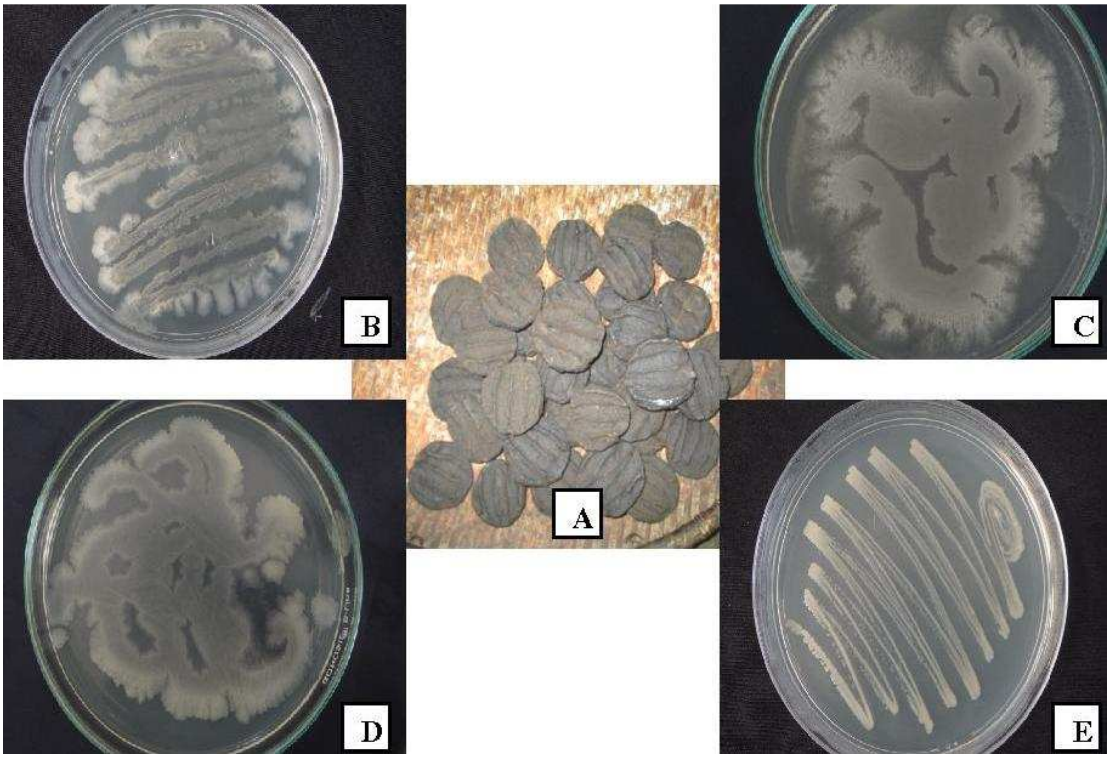


Figure - 3.3

Figure 3.3: Pure cultures of bacterial isolates from *Anishi*. **A.** *Anishi*; **B.** *Bacillus subtilis*; **C.** *Bacillus licheniformis*; **D.** *Bacillus licheniformis* and **E.** *Enterococcus faecalis*

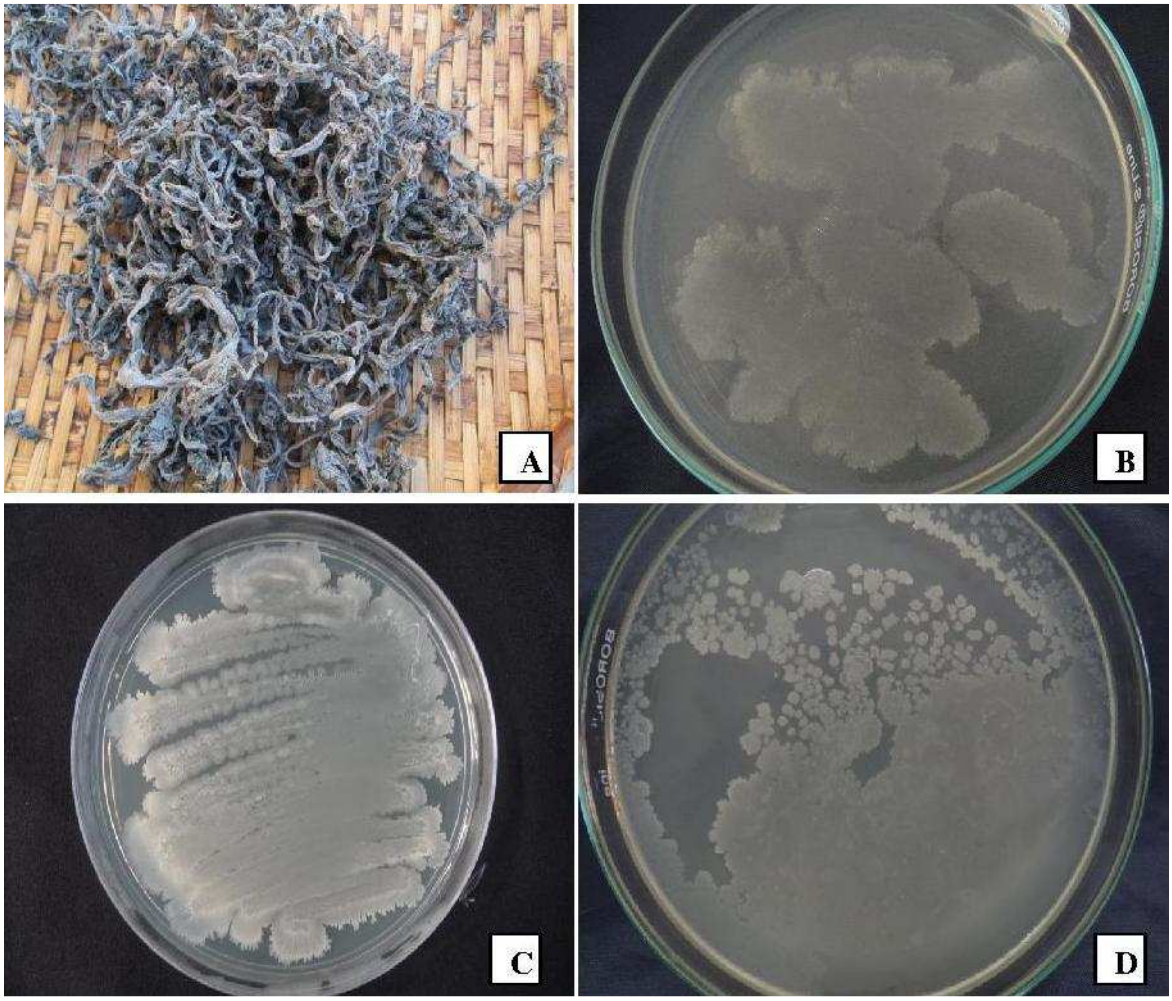


Figure - 3.4

Figure 3.4: Pure cultures of bacterial isolates from *Hungrii*. **A**. *Hungrii*; **B**. *Bacillus subtilis*; **C**. *Bacillus Licheniformis* and **D**. *Bacillus pumilus*

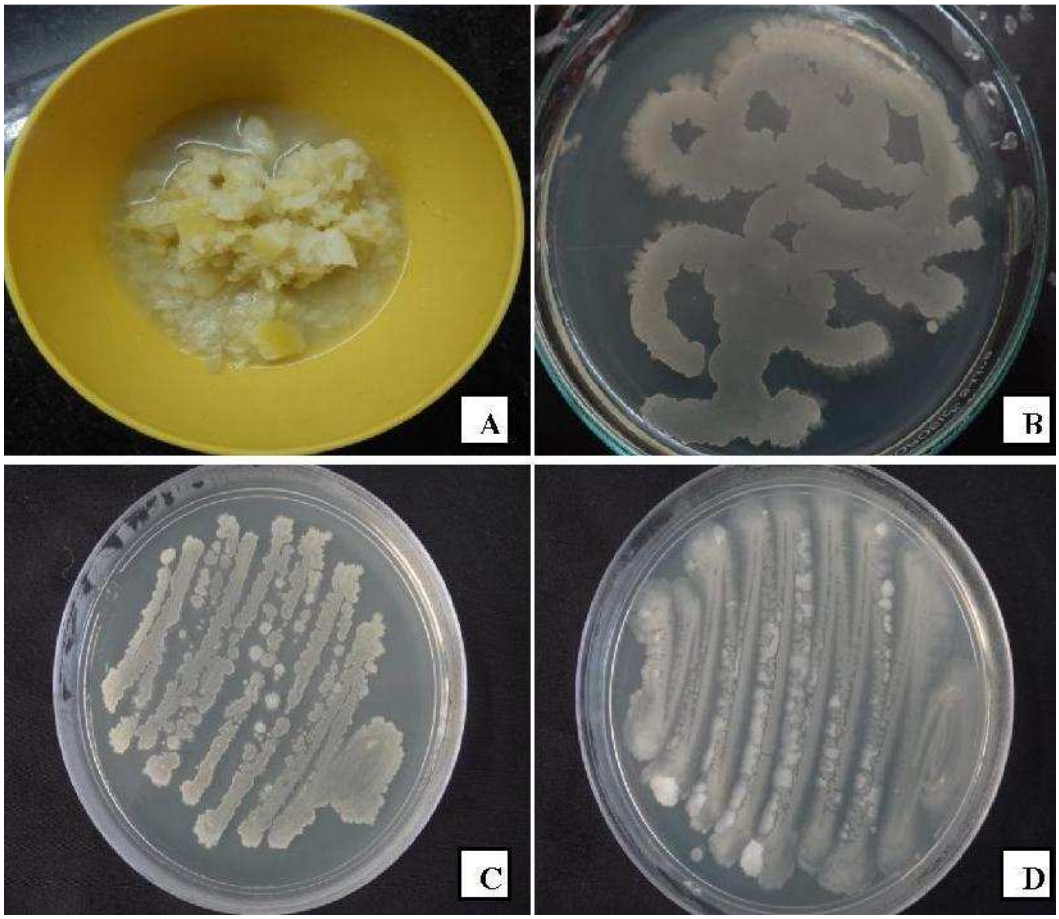


Figure - 3.5

Figure 3.5: Pure cultures of bacterial isolates from *Rhujuk/Bastanga*. **A**. *Rhujuk/bastanga*; **B.** *Bacillus subtilis*; **C**. *Bacillus licheniformis* and **D**. *Bacillus subtilis*

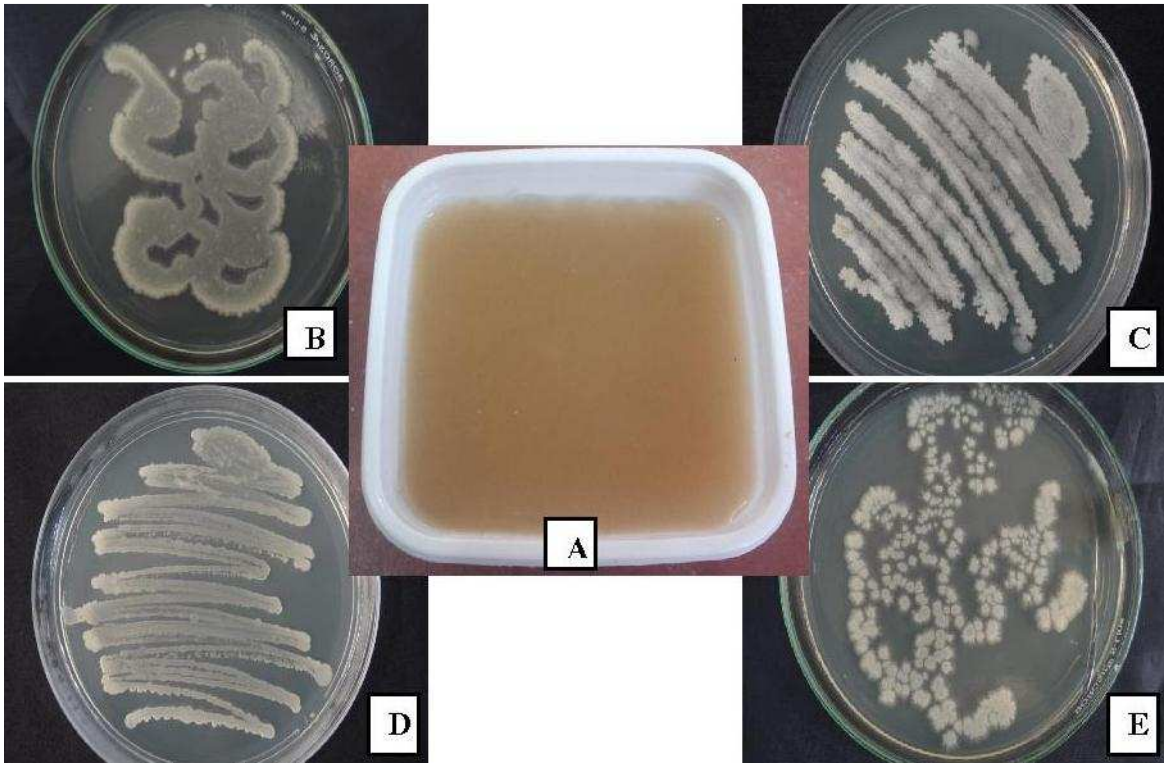


Figure - 3.6

Figure 3.6: Pure cultures of bacterial isolates from *Tsutüocie*. **A.** *Tsutüocie*; **B.** *Bacillus subtilis*; **C.** *Bacillus licheniformis*; **D.** *Bacillus pumilus* and **E.** *Bacillus licheniformis*

Table 3.6: 16S rRNA sequence based identification of microbes from *axone/akhuni* with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-2	*Bacillus licheniformis*	2455	100	0.0	99	KU301334
2	BJ-DEBCR-33	*Bacillus licheniformis*	1700	99	0.0	99	MF487831
3	BJ-DEBCR-3	*Bacillus subtilis*	2407	99	0.1	99	KU301335
4	BJ-DEBCR-24	*Bacillus subtilis*	1251	100	0.0	99	MF487822
5	BJ-DEBCR-22	*Bacillus cereus*	1094	99	0.1	99	KX364205
6	BJ-DEBCR-29	*Bacillus cereus*	1146	99	0.0	99	MF487826
7	BJ-DEBCR-1	*Staphylococcus epidermis*	2615	100	0.0	99	KU301333
8	BJ-DEBCR-21	*Alcaligenes faecalis*	2536	100	0.0	100	KX364204

Table 3.7: 16S rRNA sequence based identification of microbes from *anishi* with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-6	*Bacillus subtilis*	1931	98	0.0	99	KU854954
2	BJ-DEBCR-40	*Bacillus subtilis*	1254	99	0.0	99	MF487838
3	BJ-DEBCR-4	*Bacillus licheniformis*	1205	100	0.0	99	KU30136
4	BJ-DEBCR-17	*Bacillus licheniformis*	1055	96	0.0	99	KU854963
5	BJ-DEBCR-18	*Bacillus licheniformis*	1053	95	0.0	98	KU854964
6	BJ-DEBCR-28	*Bacillus licheniformis*	1609	100	0.0	99	MF487826
7	BJ-DEBCR-41	*Bacillus licheniformis*	1639	99	0.0	99	MF487839
8	BJ-DEBCR-20	*Bacillus pumilis*	1341	100	0.0	100	KX258616
9	BJ-DEBCR-16	*Enterococcus faecalis*	1694	98	0.0	99	KU854962

Table 3.8: 16S rRNA sequence based identification of microbes from *hungrii* with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-11	*Bacillus pumilis*	1249	94	0.0	99	KU301334
2	BJ-DEBCR-19	*Bacillus licheniformis*	1400	100	0.0	100	KX258615
3	BJ-DEBCR-26	*Bacillus licheniformis*	1290	99	0.0	98	MF487824
4	BJ-DEBCR-36	*Bacillus licheniformis*	1400	100	0.0	100	MF487834
5	BJ-DEBCR-23	*Bacillus subtilis*	1773	99	0.0	99	MF487821
6	BJ-DEBCR-35	*Bacillus amyloliquefaciens*	1469	98	0.0	98	MF487833

Table 3.9: 16S rRNA sequence based identification of microbes from *rhujuk/bastanga* with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-9	*Bacillus subtilis*	953	91	0.0	98	KU854957
2	BJ-DEBCR-14	*Bacillus subtilis*	1615	99	0.0	99	KU854961
3	BJ-DEBCR-30	*Bacillus subtilis*	1177	99	0.0	99	MF487828
4	BJ-DEBCR-31	*Bacillus subtilis*	2383	100	0.0	99	MF487829
5	BJ-DEBCR-37	*Bacillus subtilis*	985	96	0.0	97	MF487835
6	BJ-DEBCR-39	*Bacillus subtilis*	1764	100	0.0	99	MF487837
7	BJ-DEBCR-5	*Bacillus licheniformis*	1945	99	0.0	99	KU301337
8	BJ-DEBCR-38	*Bacillus licheniformis*	1184	99	0.0	97	MF487836
9	BJ-DEBCR-32	*Bacillus amyloliquefaciens*	1070	100	0.0	99	MF487830
10	BJ-DEBCR-27	*Staphylococcus pasteuri*	1646	96	0.0	99	MF487825

Table 3.10: 16S rRNA sequence based identification of microbes from *tsutuocie* with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-7	*Bacillus subtilis*	2135	98	0.0	98	KU301335
2	BJ-DEBCR-8	*Bacillus subtilis*	1591	99	0.0	99	KU854956
3	BJ-DEBCR-25	*Bacillus subtilis*	1212	100	0.0	99	MF487823
4	BJ-DEBCR-10	*Bacillus licheniformis*	1116	91	0.0	98	KU854958
5	BJ-DEBCR-12	*Bacillus pumilis*	1458	96	0.0	98	KU854960
6	BJ-DEBCR-34	*Bacillus pumilis*	1692	94	0.0	99	MF487832

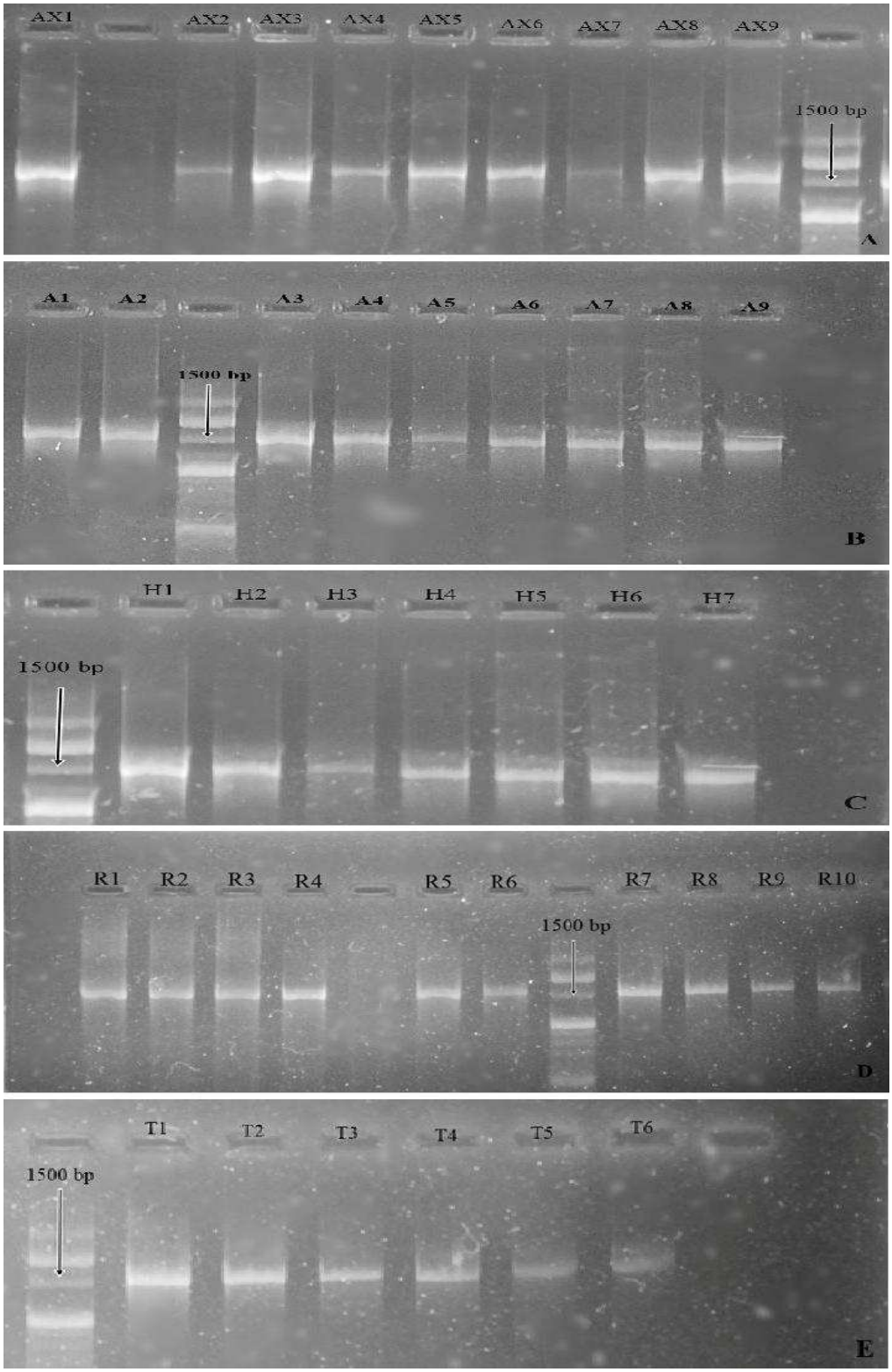


Figure - 3.7

Figure 3.7: PCR amplification of 16S rRNA region**. A.** *Axone/akhuni***; B.** *Anishi***; C.** *Hungrii***; D.** *Rhujuk/bastanga* and **E.** *Tsutuocie*

Colonies isolation and DNA sequence analysis

The 16S rRNA gene is very useful because the genome of all the bacteria contains this conserved gene and any small variability in this region is unique and specific to each species. This characteristic is usually harnessed in their identification. In this study, species-level identification was primarily based on 16S rRNA gene sequences. Microbial species present in *axone/akhuni, anishi, hungrii, rhujuk/bastanga* and *tsutuocie* identified after comparing their sequence data to sequences listed in the NCBI database are given in **table 3.6 to 3.10.** The PCR product of 16S rRNA gene gave an approximate 1500 bp amplicon **(Figure 3.7).**

Axone/akhuni

Identification results of the microbial strains isolated from *anishi* are listed in **table 3.6.** Total microbial load was in the range of 10^7cfu ml^{-1}. Pure culture colony of the observed bacteria having different morphology and colour are given in **figure 3.2**. Most of the isolates were gram negative, spore formers and catalase positive belonging to the genus *Bacillus*. The different groups of *Bacillus* sp. identified by sequencing the partial 16S rRNA were *Bacillus subtilis, Bacillus licheniformis* and *Bacillus cereus*. The two isolates BJ-DEBCR-3 and BJ-DEBCR-24 showed 99% similarity to *Bacillus subtilis* deposited in the GenBank based on their 16S rRNA gene sequences. Isolates BJ-DEBCR-2 and BJ-DEBCR-33 after sequencing of the 16S rRNA gene showed similarity to *Bacillus licheniformis* (99%) in GenBank sequences. Isolates BJ-DEBCR-22 and BJ-DEBCR-29 were found to have 99% similarity with *Bacillus cereus*. One of the isolate BJ-DEBCR-21 was found to be gram negative rod which was identified by sequencing the partial 16S rRNA, having 100% similarity with *Alcaligene faecalis*. Another isolate BJ-DEBCR-1 was found to be gram positive cocci, which was identified as belonging to

Staphylococcus epidermis based on 16S rRNA gene sequencing (99% similarity to GenBank sequences) **(Appendix-III).**

Anishi

Identification results of the microbial strains isolated from *anishi* are listed in **table 3.7.** Total microbial loads were in the range of 10^4 cfu ml^{-1}. Pure culture colony of the observed bacteria having different morphology and colour are given in **figure 3.3**. Most of the isolates were gram negative, spore formers and catalase positive belonging to the genus *Bacillus*. The different groups of *Bacillus* spp. identified by sequencing the partial 16S rRNA were *Bacillus subtilis* and *Bacillus licheniformis*. Two of the isolates BJ-DEBCR-6 and BJ-DEBCR-40 were found to have 99% similarity with *Bacillus subtilis*. Isolates BJ-DEBCR-18, BJ-DEBCR-17, BJ-DEBCR-28, BJ-DEBCR-41 and BJ-DEBCR-4 showed 98-99% similarity to *Bacillus licheniformis* deposited in the GenBank based on their 16S rRNA gene sequences. Isolate BJ-DEBCR-20 was identified as *Bacillus pumilis* showing 100% homology to GenBank sequences, respectively.One of the isolate BJ-DEBCR-16 was found to be gram negative rod which was identified as belonging to *Enterococcus faecalis* based on 16S rRNA gene sequencing (99% similarity to GenBank sequences) **(Appendix-III).**

Hungrii

Identification results of the microbial strains isolated from *hungrii* are listed **in table 3.8.** Total microbial loads were in the range of 10^4 cfu ml^{-1}. Pure culture colony of the observed bacteria having different morphology and colour are given in **figure 3.4**. All the isolates were gram negative, spore formers and catalase positive belonging to the genus *Bacillus*. The different groups of *Bacillus* sp. identified by sequencing the partial 16S rRNA were *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilis* and *Bacillus amyloliquefaciens.* Isolate BJ-DEBCR-23 was found to have 99% similarity with *Bacillus subtilis*. Three isolates BJ-DEBCR-26, BJ-DEBCR-19 and BJ-DEBCR-36 showed 98-

100% similarity to *Bacillus licheniformis* deposited in the GenBank based on their 16S rRNA gene sequences. Isolates BJ-DEBCR-11 and BJ-DEBCR-35 after sequencing of the 16S rRNA gene showed similarity to *Bacillus pumilis* (99%) and *Bacillus amyloliquefaciens* (98%) in GenBank sequences **(Appendix-III).**

Rhujuk/bastanga

Identification results of the microbial strains isolated from *rhujuk/bastanga* are listed **in table 3.9.** Total microbial loads were in the range of 10^7 cfu ml^{-1}. Pure culture colony of the observed bacteria having different morphology and colour are given in **figure 3.5**. Most of the isolates were gram negative, spore formers and catalase positive belonging to the genus *Bacillus*. The different groups of *Bacillus* spp. identified by sequencing the partial 16S rRNA were *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens.* . Six isolates BJ-DEBCR-37, BJ-DEBCR-9, BJ-DEBCR-14, BJ-DEBCR-30, BJ-DEBCR-31 and BJ-DEBCR-39 showed 97-99% similarity to *Bacillus subtilis* deposited in the GenBank based on their 16S rRNA gene sequences. Isolates BJ-DEBCR-38 and BJ-DEBCR-5 were found to have 97-99% similarity with *Bacillus licheniformis*. Isolate BJ-DEBCR-32 was found to have 99% similarity with *Bacillus amyloliquefaciens*. One of the isolate BJ-DEBCR-27 was found to be gram negative rod which was identified as belonging to *Staphylococcus pasteuri* based on 16S rRNA gene sequencing (99% similarity to GenBank sequences) **(Appendix-III).**

Tsutuocie

Identification results of the microbial strains isolated from *tsutuocie* are listed in **table 3.10.** Total microbial loads were in the range of 10^4 cfu ml^{-1}. Pure culture colony of the observed bacteria having different morphology and colour are given in **figure 3.6.** All the isolates were gram negative, spore formers and catalase positive belonging to the

genus *Bacillus*. The different groups of *Bacillus* sp. identified by sequencing the partial 16S rRNA were *Bacillus subtilis, Bacillus licheniformis* and *Bacillus pumilis.* Three of the isolates BJ-DEBCR-7, BJ-DEBCR-8 and BJ-DEBCR-25 showed 98-99% similarity to *Bacillus subtilis* deposited in the GenBank based on their 16S rRNA gene sequences. Isolate BJ-DEBCR-10 after sequencing of the 16S rRNA gene showed similarity to *Bacillus licheniformis* (98%) and isolates BJ-DEBCR-12 and BJ-DEBCR-34 showed similarity to *Bacillus pumilis* (98-99%) in GenBank sequences **(Appendix-III).**

***p*H**

Microorganism needs particular environment to survive and grow, thus the *p*H value is an important factor upon which microbial population can be determined. The *p*H levels of *anishi, hungrii* and *rhujuk/bastanga* were observed to be acidic with *p*H value of 5.8, 5.2 and 4.7 respectively. Whereas, *axone/akhuni* and *tsutuocie* were found to be alkaline in nature with a *p*H value of 8 and 8.2 respectively.

Phylogenetic analysis

The 16S rRNA sequencing has become a useful tool in the study of phylogenetic relationships between microorganisms and in identifying taxonomic position of the unknown isolate. Such relationships and taxonomic positions are normally illustrated in phylogenetic tree.

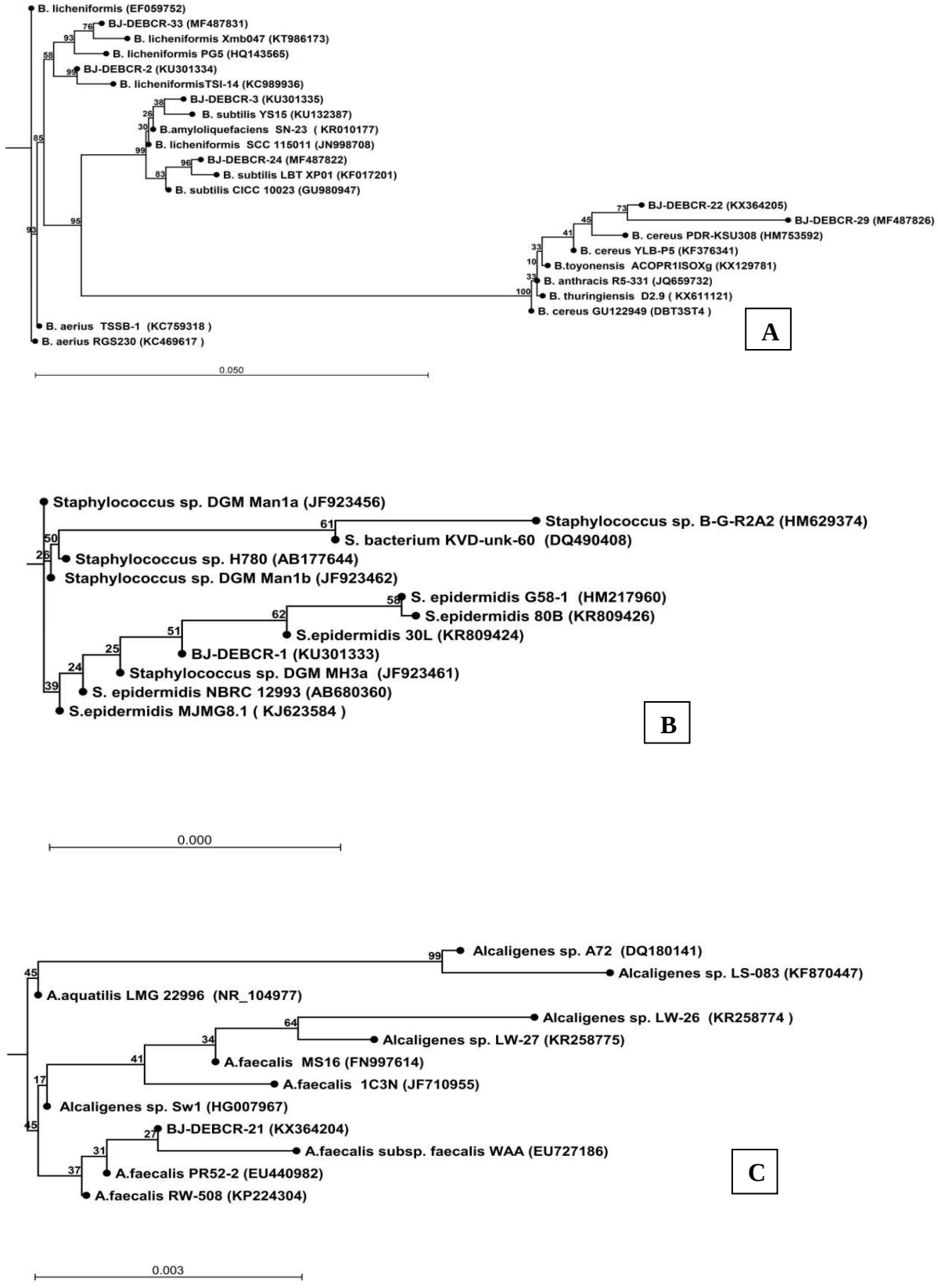


Figure 3.8: Phylogenetic tree showing relationships of isolate and related species from NCBI. A) *Bacillus* species. B) *Staphylococcus epidermis*. C) *Alcaligenes faecalis*

Axone/akhuni

Neighbour-joining phylogenetic tree was constructed for isolates of *Bacillus* species using the closest related sequences from NCBI GenBank as reference strains. It was observed that three 16S rRNA phylogenetic groups were formed. The strains BJ-DEBCR-33 and BJ-DEBCR-2 grouped with *Bacillus licheniformis* reference strains. Strains BJ-DEBCR-3 and BJ-DEBCR-24 grouped with *Bacillus subtilis* reference strains and the other two strains BJ-DEBCR-22 and BJ-DEBCR-29 were grouped with *Bacillus cereus* reference strains. Phylogenetic tree constructed for the strain BJ-DEBCR-1, showed forming a group with *Staphylococcus epidermis* reference strain. Another strain BJ-DEBCR-21 was found to form a group with *Alcaligenes faecalis* reference strain **(Figure 3.8 A, B, C).**

Anishi

Neighbour-joining phylogenetic tree was constructed for isolates of *Bacillus* species using the closest related sequences from NCBI genbank as reference strains. It was observed that three 16S rRNA phylogenetic groups were formed. The strains BJ-DEBCR-40 and BJ-DEBCR-6 grouped with *Bacillus subtilis* reference strains. Strains BJ-DEBCR-17, BJ-DEBCR-4, BJ-DEBCR-18 and BJ-DEBCR-41 grouped with *Bacillus licheniformis* reference strains and the other strain BJ-DEBCR-20 grouped with *Bacillus pumilis* reference strain. Phylogenetic tree constructed for the strain BJ-DEBCR-16, showed forming a group with *Enterococcus faecalis* reference strain **(Figure 3.9 A, B).**

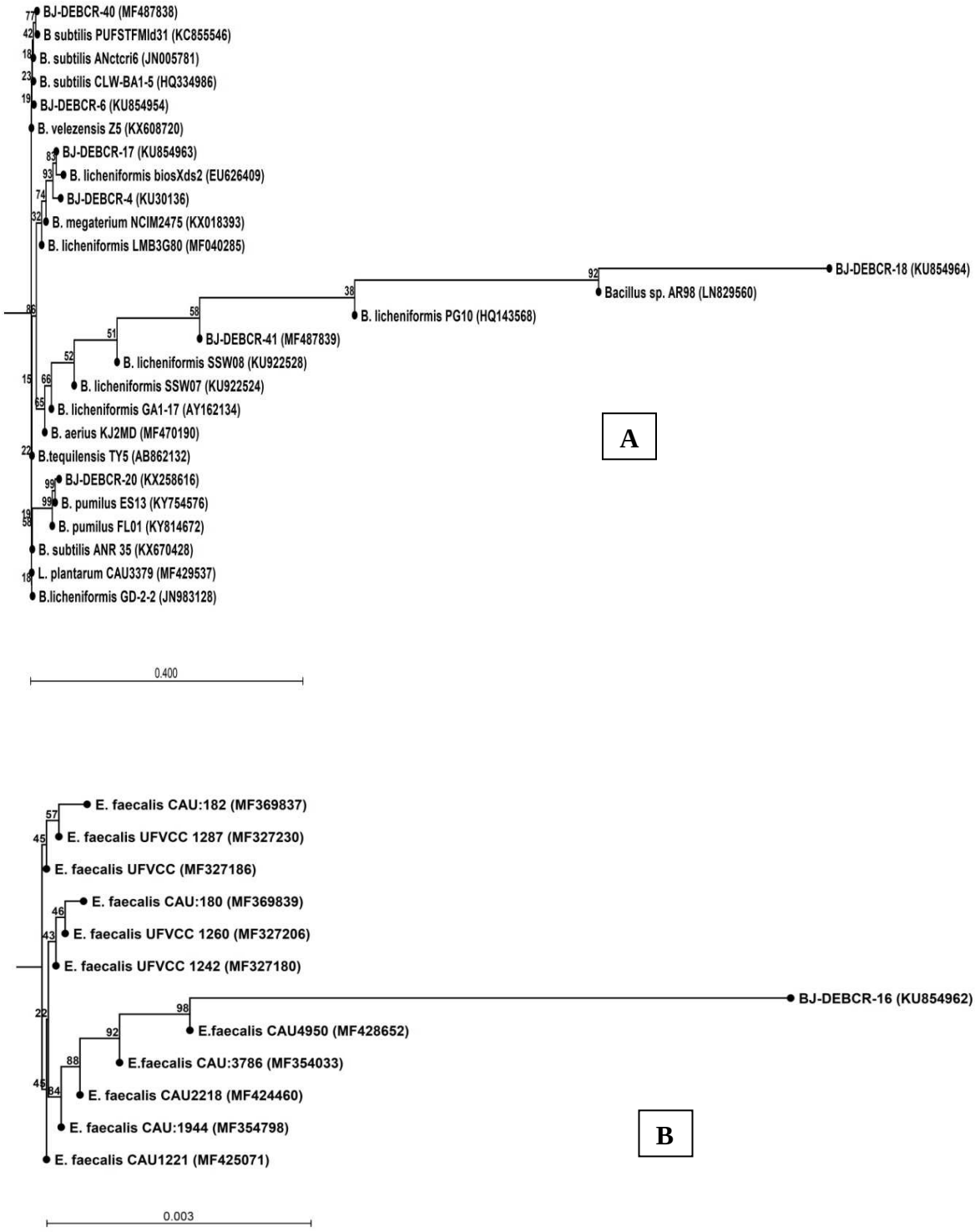


Figure 3.9: Phylogenetic tree showing relationships of isolate and related species from NCBI obtained from *anishi.* A) *Bacillus* species. B) *Enterococcus faecalis*

Hungrii

Neighbour-joining phylogenetic tree was constructed for isolates of *Bacillus* species using the closest related sequences from NCBI genbank as reference strains. It was observed that four 16S rRNA phylogenetic groups were formed. The strain BJ-DEBCR-23 grouped with *Bacillus subtilis* reference strain. Strains BJ-DEBCR-36, BJ-DEBCR-19 and BJ-DEBCR-26 grouped with *Bacillus licheniformis* reference strains. The strain BJ-DEBCR-11 was grouped with Bacillus pumilis reference strain and the other strain BJ-DEBCR-35 was grouped with *Bacillus amyloliquefaceins* reference strain **(Figure 3.10).**

Rhujuk/bastanga

Neighbour-joining phylogenetic tree was constructed for isolates of *Bacillus* species using the closest related sequences from NCBI genbank as reference strains. It was observed that four 16S rRNA phylogenetic groups were formed. The strains BJ-DEBCR-38 and BJ-DEBCR-5 grouped with *Bacillus licheniformis* reference strains. Strains BJ-DEBCR-37, BJ-DEBCR-39, BJ-DEBCR-14 and BJ-DEBCR-30 grouped with *Bacillus subtilis* reference strains and the other strain BJ-DEBCR-32 grouped with *Bacillus amyloliquefaceins* reference strain. The strains BJ-DEBCR-9 and BJ-DEBCR-31 were grouped with *Bacillus subtilis* reference strains. Phylogenetic tree constructed for the strain BJ-DEBCR-27, showed forming a group with *Staphylococcus pasteuri* reference strain **(Figure 3.11 A, B).**

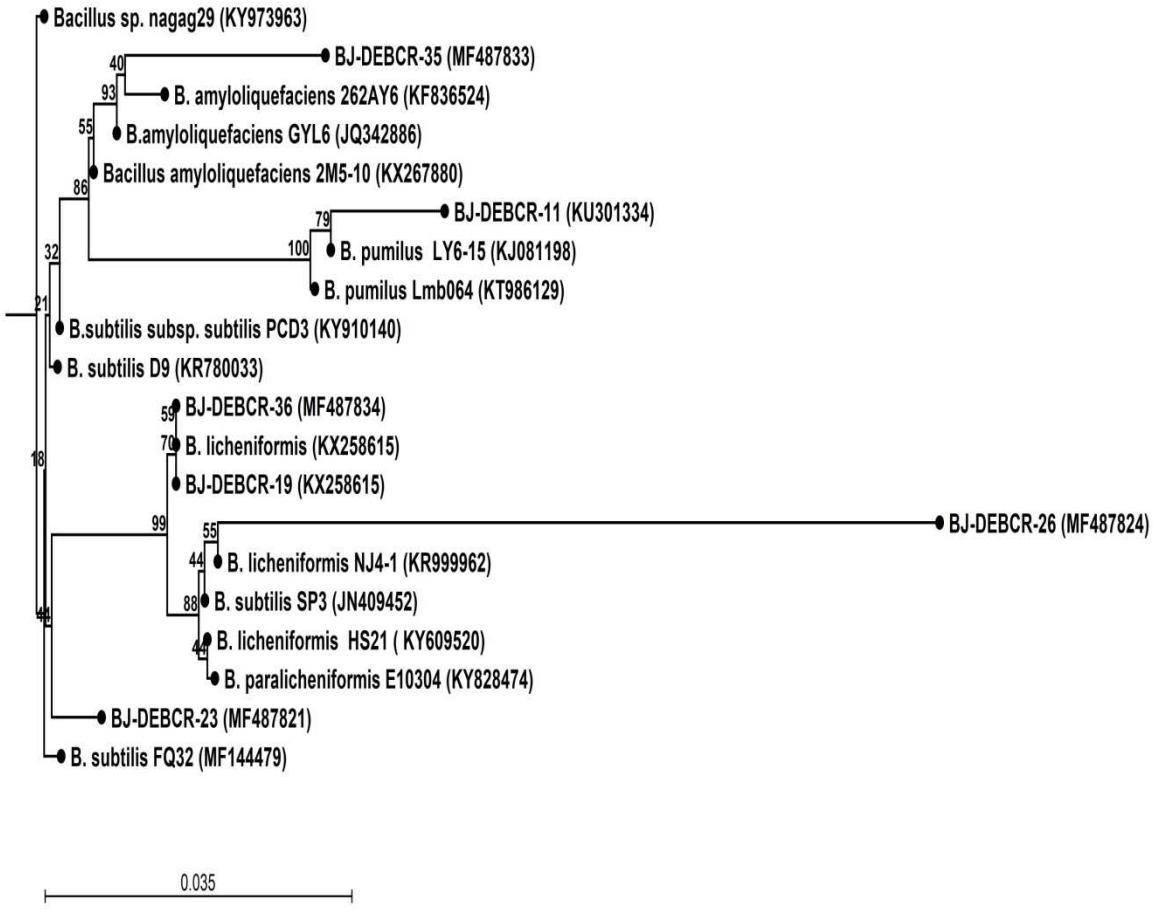


Figure 3.10: Phylogenetic tree showing relationships of isolate and related species from NCBI obtained from *hungrii*

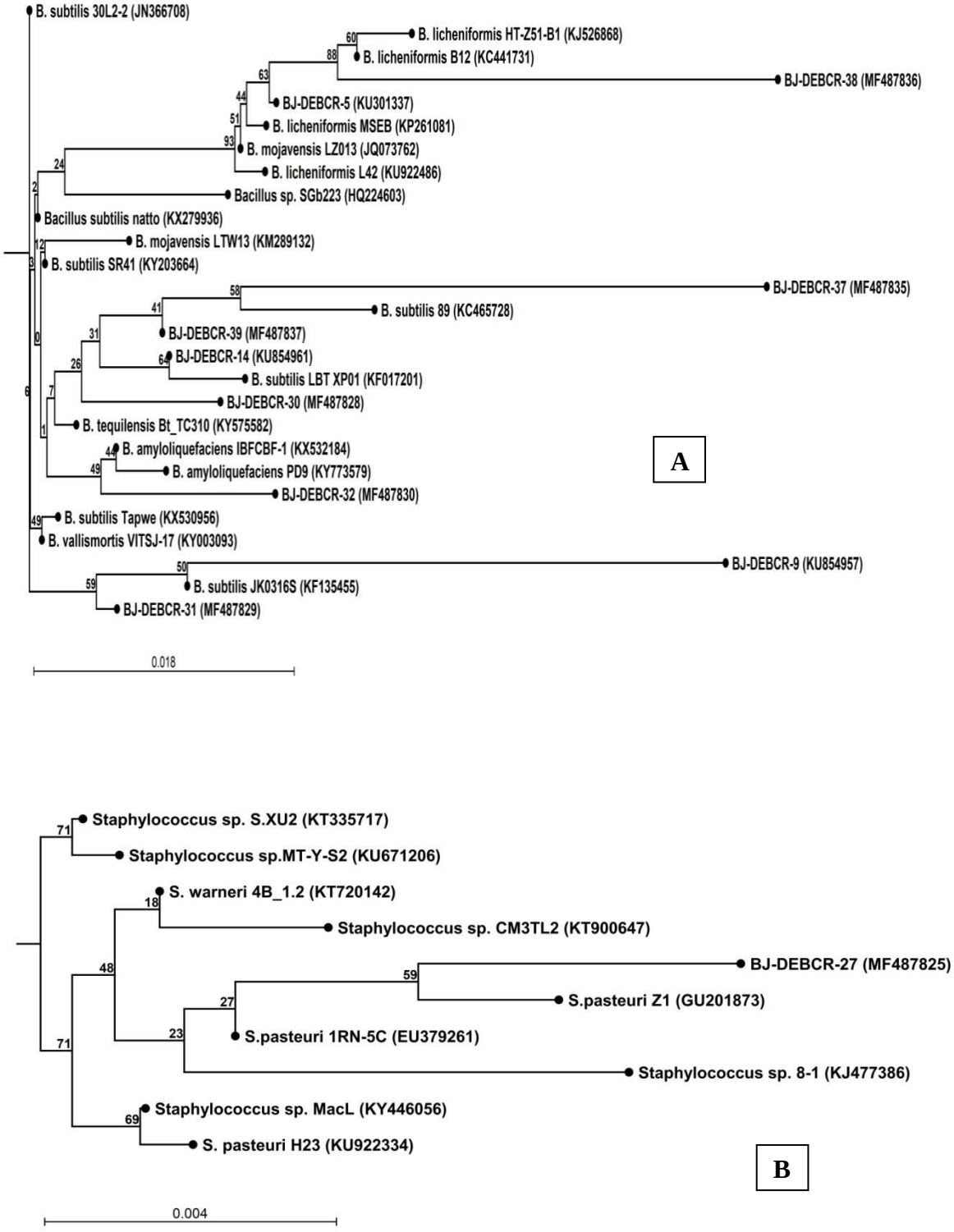


Figure 3.11: Phylogenetic tree showing relationships of isolate and related species from NCBI obtained from *rhujuk/bastanga*. A) *Bacillus* species. B) *Staphylococccus pasteuri*

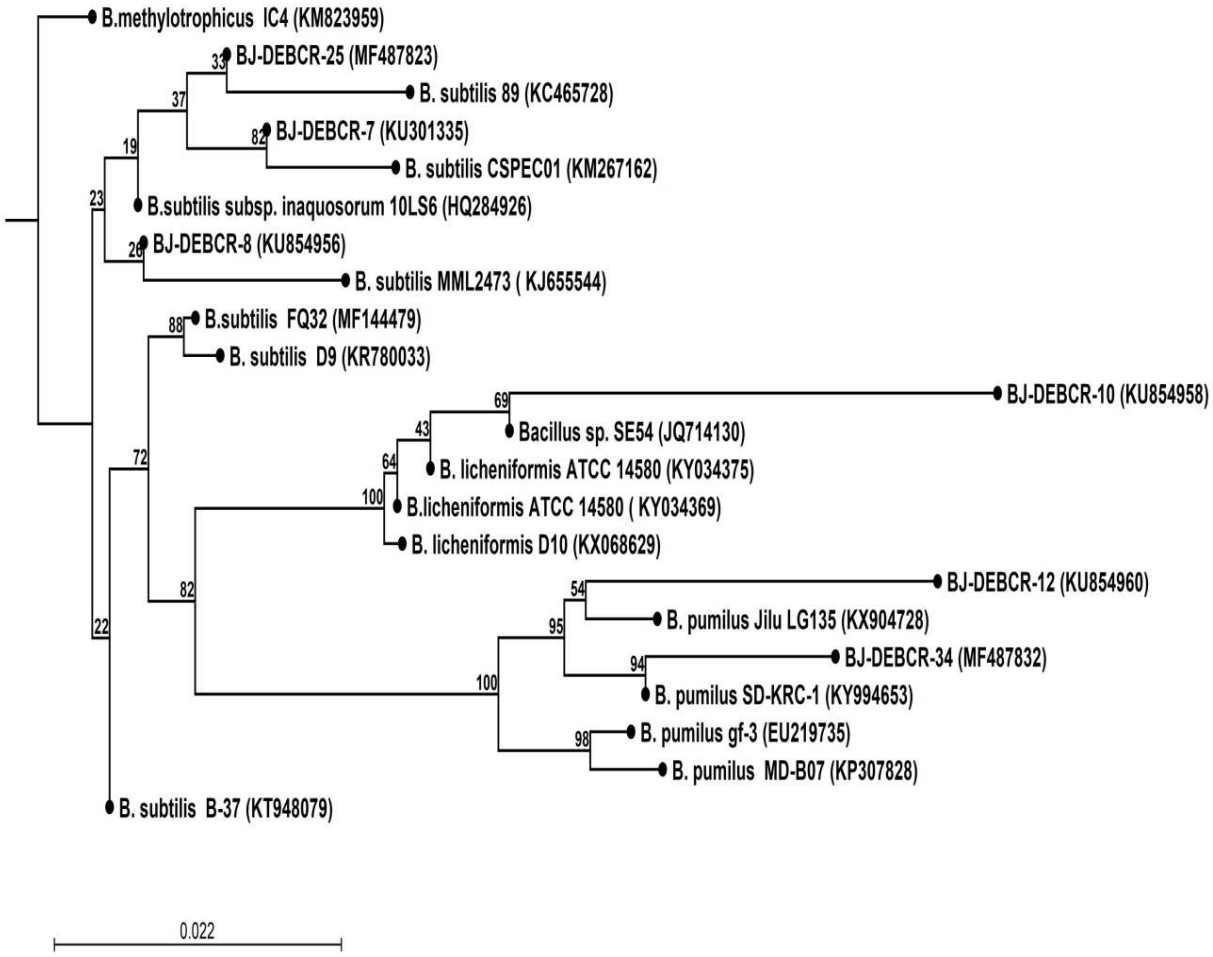


Figure 3.12: Phylogenetic tree showing relationships of isolate and related species from NCBI obtained from *tsutuocie*.

Tsutuocie

Neighbour-joining phylogenetic tree was constructed for isolates of *Bacillus* species using the closest related sequences from NCBI genbank as reference strains. It was observed that three 16S rRNA phylogenetic groups were formed. The strains BJ-DEBCR-8, BJ-DEBCR-25 and BJ-DEBCR-7 grouped with *Bacillus subtilis* reference strains. Strain BJ-DEBCR-10 grouped with *Bacillus licheniformis* reference strain and the other two strains BJ-DEBCR-12 and BJ-DEBCR-34 were grouped with *Bacillus pumilis* reference strains **(Figure 3.12).**

Discussion

Fermented food products have a long history and form significant part of the diet of many indigenous communities in the developing world. Definitive identification of microorganisms is essential for a wide variety of application, industrial, biomedical, pharmaceutical and environmental studies (Adeyemo and Onilude, 2014). Nagaland has a rich diversity of indigenous fermented foods, which so far are least explored and therefore these food items serve as rich reserve of unexplored microorganisms. The type of bacterial flora developed in each fermented food depends on the water activity, pH, salt, concentration, temperature and the composition of the food matrix (Font de Valdez et. al., 2010). Fermentation is spontaneous and uncontrolled thus resulting in a product of variable quality. Spontaneous fermentation typically results from the competitive activities of different microorganisms whereby strains best adapted and with the highest growth rate will dominate during particular stages of the process. Thus, the present study was aimed to isolate and identify the dominant microorganisms present in the final fermented product.

Preparation and consumption of sticky, non-salty, flavoursome fermented soybean foods are the traditional wisdom of the people from several South-East Asian countries, which have fostered a distinct food culture of the people (Tamang, 2010). Some of the common ethnic, nonsalted sticky fermented soybean foods are *natto* (Japan); *kinema* (India, Nepal, and Bhutan); *tungrymbai*, *bekang*, *hawaijar* and *peruyaan* (India); *thua nao* (Thailand); *chungkokjang* (Korea) (Nagai and Tamang, 2010). Sarkar et al. (1994) reported the members of *Bacillus subtilis* to be the most dominant microorganisms involved in the production of *kinema.* Besides bacilli, the other lactic acid bacterium is *Enterococcus faecium,* and two types of yeasts i.e., *Candida parapsilosis* and *Geotrichum candidum* were also isolated from *kinema* samples. Jeyaram et al. (2008) also reported *Bacillus* species to be the most predominant microorganism in *hawaijar,* identified as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus cereus*. Besides *Bacillus, Staphylococcus* sp. and few bacterial strains of *Alkaligenes* species and *Providencia rettgeri* were also reported. In this present study *Bacillus* species was found to be the most dominant microorganism present in the products and identified as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus cereus*. Although the method of production and culinary practices vary from product to product, all bacilli-fermented Asian soybean foods have a characteristic stickiness and typical flavour. However, there was no yeast or moulds detected in any of the product as reported in *kinema* (Sarkar et al., 1994). Thokchom and Joshi (2012); Chettri and Tamang (2015) reported that *Bacillus subtilis* was the dominant bacteria involved in production of *tungrymbai* and *bekang.* Furthermore, reports also show that the gram-positive, spore-forming, rod-shaped bacterium *Bacillus subtilis* is responsible for the fermentation of *thua nao* and *chungkokjang* (Sundhagul et al., 1972; Tamang et al., 2002). The presence of *Staphylococcus epidermis* and *Bacillus cereus* in *axone/akhuni* product, which are

considered as food pathogens may have entered the food from unhygienic use of tools and hands during its preparation process. *Bacillus cereus* was also previously isolated from *hawaijar* (Jeyaram et al., 2008) and *kinema* (Sarkar et al., 2002). *Bacillus cereus* is a gram-positive, rod-shaped, sporeforming foodborne pathogen. Since the spore of *B. cereus* is resistant to heat, acid, ultraviolet, gamma irradiation, water stress, it is widely isolated from soil, sediment, dust, plants, and food production environments (Krasowska et al., 2015). Thokchom and Joshi (2012) and Jeyaram et al. (2008) also reported the presence of food pathogen *Staphylococcus* species isolated from *tungrymbai* and *hawaijar*, which normally find their way into fermented products from the raw material, personnel, animal skins and the environment. *Alcaligenes* species was also identified from the present study, which was also reported from Hawaijar (Jeyaram et al., 2008). *Alcaligenes* species are mostly found in soil or water (Kesik et al., 2006).

Bacillus species are reported to be dominant in soya fermented foods and their role being to accelerate the hydrolysis of protein, thus releasing ammonia (Chukeatirote, 2015). The release of ammonia is responsible for the ammoniacal odour characteristic of most soybean based fermentations (Sarkar et al., 1993). The prevalence of *Bacillus* species in the fermented product may be due to the alkaline condition (*p*H 8) that occurs during the fermentation process leading to favourable condition for some bacteria to grow, but also causing unfavourable condition for other microbes to grow. The rise in *p*H during production of these foods is due to the ability of the dominant microorganisms, *Bacillus* species to hydrolyse proteins into amino acids and ammonia (Parkouda et al, 2009). The preservative and flavour characteristics of alkaline fermenters are derived in part from the liberation of ammonia and increased *p*H (Beaumont, 2002). Use of *Bacillus subtilis* as a pure starter culture reported high increase in total amino acids and isoflavones in soybean fermented product (Shon et al., 2007; Moktan et al., 2008;

Chonkeeree et al., 2013). Fujita et al. (1993) isolated *Bacillus subtilis* also known as *Bacillus natto* from *natto,* whose cells produce many enzymes, vitamin K2 and protease, subtilisin, which can degrade soybean allergens and shows fibrinolytic activity. Amoa-Awua et al. (2006) also reported that soy-*daddawa* is produced by the fermentation of bacteria notably *Bacillus* species. The *Bacillus* species produced extracellular enzymes which hydrolysed the organic components of the fermenting beans resulting in increased soluble products especially free amino acids. Dajanta et al. (2009) also reported the increase in levels of daidzein and genistein in *thua nao* soybean product inoculated with *Bacillus subtilis* strain. Chettri and Tamang (2015) reported high production of Poly-γ-glutamic acid (PGA) in *tungrymbai* and *bekang* inoculated with *Bacillus subtilis* (TSS1:B25 and BT: B9) strain. Similarly, Shin and Jeong (2015) also reported increase in levels of dietary fiber, phosphatide, isoflavone, phenolic acid, poly-glutamic acid (PGA) and saponine in *chongkukjang* fermented with *Bacillus subtilis*. Apart from *Bacillus subtilis* strain, *Bacillus licheniformis* strain was also reported to produce good quality *chongkukjang* fermented product (Kim et al., 2004; Baek et al., 2010).

Fermented food products prepared from leafy vegetables for bio-preservation to extend the storage life and enhance safety of foods using the natural microflora, is popularly practiced in Nagaland. These products are mostly non-salted and are either sun dried or baked at high temperature after the completion of the fermentation period. The *p*H of *anishi* and *hungrii* was recorded to be 5.8 and 5.2 respectively, which renders it to be acidic. Production of organic acids and lactic acids by *Bacillus* species have been reported by Ohara and Yahata (1996) and Yan et al. (2012) .In the present study, the average microbial load in *anishi* and *hungrii* was found to be low i.e., 10^4 cfu ml^{-1}. It may be due to implication of post fermentation technique like sun drying or exposing of the product to high temperature, making it difficult for many bacteria and moulds to grow

(Steinkraus, 2002). Studies have reported the presence of lactic acid bacteria in most of the vegetable based fermented food products (Lee and Kang, 2004; Tamang et al., 2005; Breidt et al., 2013). However, in the present study the most dominant microorganisms in *anishi* were reported to belong to the members of the *Bacillus* species, which were identified as *Bacillus subtilis* and *Bacillus licheniformis*, after comparing with 16S rRNA sequences from the NCBI genbank. Another bacteria isolated was identified as *Enterococcus faecalis*. The presence of a bacterial species representing *E. faecalis* is responsible for sensory characteristics of the final product, as this species is often prevalent in foods (Giraffa, 2003; Gomes et al., 2008). In addition, some *Enterococci* strains, especially *E. faecalis* and *E. faecium* may produce bacteriocins that are active against a plethora of food borne pathogens (Toit et al., 2000), making them suitable candidates for controlling emerging pathogens during food fermentation (Callewaert et al., 2000). Despite the safety and pleasant sensory attributes imparted by *E. faecalis* in foods, some strains of *E. faecalis* and *E. faecium* are associated with infection that pose challenges to food safety (Gomes et al., 2008). Similarly, in *hungrii* the most dominant microorganisms isolated belonged to *Bacillus* species, which were identified as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilis* and *Bacillus amyloliquefaciens*. *Bacillus amyloliquefaciens* is one of most prevalent gram-positive aerobic spore-forming bacteria with the ability to synthesize polysaccharides and polypeptides (Geng et al., 2011). The antifungal property of *Bacillus licheniformis* is reported by Patel et al. (2004) and Tendulkar et al. (2008). Bottone and Peluso (2003) reported a compound produced by *Bacillus pumilus* (MSH) that inhibits *Mucoraceae* and *Aspergillus* species. The presence of *Bacillus* species in such foods can be linked to different factors such as post fermentation method of baking at high temperature or drying during the process, which

select for heat resistant microorganisms, especially spore forming bacteria (Quoba et al., 2008).

Bamboo shoots constitute a major component of traditional cuisine in most of the Asian countries. It forms a rich ecological niche which harbours a plethora of microorganisms (Thakur et al., 2016). Mostly, the production of fermented bamboo shoots involves the natural fermentation process with various lactic acid bacteria playing dominant role in imparting flavour, taste and aroma to the product. *Lactobacillus plantarum, L. brevis, L. casei, L. fermentum, L. curvatus, Leuconostoc mesenteroides, L. fallax* and *Tetragenococcus halophilus* are predominantly found in fermented shoots (Nongdam and Tikendra, 2014). However, in the present study *bastanga* had microbial load in the range of 10^7 cfu ml^{-1}, which was mostly dominated by *Bacillus* species. The different strains identified were *Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens.* Similarly, Sarangthem and Singh (2003) and Jeyaram et al. (2010) also isolated *Bacillus subtilis* and *Bacillus licheniformis*, from fermented bamboo shoot product *soibum*. They also reported that *Bacillus subtilis* showed highest level of efficiency in accumulation of total phytosterol, which are the precursors of many pharmaceutically active steroids, during fermentation. Tamang et al. (2012) also reported the presence of *Bacillus subtilis* and other *Bacillus* species like *Bacillus circulans, Bacillus firmus* and *Bacillus sphaericus* from *tuaithur,* a fermented bamboo shoot product of Assam. Bioconversion ability of *Bacillus* sp. found as metabolites in fermented succulent shoots of bamboo makes them ideal source of bioactive compounds like phytosterols (precursors of many pharmaceutically active steroids) (Wu et al., 2015). Another bacteria isolated from *bastanga* was identified as *Staphylococcus* species. The presence of *Staphylococcus* species in food products is generally undesirable especially when the count is greater than 10^2 cfu g^{-1}. Low numbers of these organisms is indicative

of poor handling conditions whereas high counts are frequently associated with incidences of food poisoning (Flint et al., 2005).

Lactic acid fermentation usually plays a very important role in cucumber fermentation. Most commercial cucumber fermentations rely upon growth of the microorganisms that is naturally present on the surface of cucumbers (Breidt et al., 2013). Cucumbers are mostly fermented by adding salt or acetic acid to limit the growth of spoilage microorganisms (Gates and Costilow, 1981). However, during the preparation of *tsutuocie* no salt is added but instead water was added. In the present study, the microbial load in *tsutuocie* was in the range of 10^4 cfu ml^{-1}, the microorganisms were found mostly to be *Bacillus* species. They were *Bacillus subtilis, Bacillus licheniformis* and *Bacillus pumilis*. Munimbazi and Bullerman (1998) described antifungal metabolites produced by *Bacillus pumilus*, which inhibited mycelial growth (spore germination) of *Aspergillus, Penicillium* and *Fusarium* species, and aflatoxin production by *Aspergillus parasiticus*. *Tsutuocie* was found to be alkaline in nature with a *p*H as high as 8.2. This high *p*H of the fermented product might have been the reason for the occurrence of *Bacillus* species in dominance over other microorganisms in the final product. The absence of LAB in most of the fermented vegetables and fruit, probably owes to loss of survival during aging process (Pederson and Ward, 1979; Lindgren and Dobrogosz, 1990). These results are also in agreement with the generally accepted concept that traditional fermentations are dominated by a few microbial species that are selected during the course of fermentation because of good adaptation to the food matrix (Hounhouigan et al., 1993; Halm et al., 1996). LAB involved in vegetable fermentation were not detected, which may be due to dominance of Non-LAB over fastidious LAB and further it is also reported that fresh fruits and vegetables harbour only 0.1% of LAB and 99.9% Non-LAB (Montet et al., 1999) and hence may have gone undetected.

Bacillus species was found to be predominant microflora in these fermented food items, indicating their versatility and easy access in the manufacturing process but it is reported that they are not involved in spoilage instead some of the *Bacillus* species prevent fungal growth (Tendulkar et al., 2008; Schnurer and Magnusson, 2005; Perez et al., 2017). Yeast and moulds were absent in the fermented products which further indicated that growth of enteric bacteria have predominantly created unfavourable conditions (especially *p*H) for growth of fungi (Pitt and Hocking, 2009). *Bacillus* species produce about 167 biological compounds active against bacteria, fungi, protozoa and viruses (Bottone and Peluso, 2003). In addition to lactic acid bacteria, it is known that *Bacillus* members do possess probiotic activity (Li et al., 2004; Li et al., 2007; Bates et al., 2008). Shahcheraghi et al. (2015) reviewed the various potential of *Bacillus subtilis* strains as a probiotic, producing antibiotics and enzymes that are important in both medical and industrial sciences. Studies have reported that *Bacillus* species possess wide range of inhibitory spectrum against pathogenic bacteria due to secretion of antimicrobial compounds viz. bacteriocins (Sharma et al., 2013).

In this present study, the use of a combination of both culture-dependent and culture independent method resulted in identification of strains that were previously isolated from other fermented food products. However, some strains that were isolated in this study could not be detected in the previous studies. This may be due to the bacterial strains entering a viable but non-cultivable state, characterized by metabolically active cells that do not produce colonies on both selective and non-selective media (Giraffa and Neviani, 2001), which illustrates one of the main advantages of culture-independent approaches over culture-dependent methods (Muyzer and Smalla, 1998; Giraffa and Neviani, 2001).

Summary and Conclusion

Each fermented food is associated with a unique group of microorganisms which increases the concentration of protein, essential amino acids, fatty acids, vitamins, and the availability of minerals (Dahal et al., 2005). In Nagaland, fermented food products are still produced from spontaneous fermentation. Consequently, there is immense variation in the microorganism involved as well as sensory characteristics and quality of the fermented products. Present study revealed the presence of *Bacillus* species in almost all the fermented food products studied for its microbial population. *Bacillus* species are reported to be spore formers and are capable of surviving even in adverse conditions, which may be one of the reasons for their dominance in the final product. *Bacillus* species are also reported to be present in the raw materials, which might have been incorporated in the fermented product during the fermentation process. The average microbial load in fermented products like *anishi, hungrii* and *tsutuocie* were low, which may be due to the pre or post fermentation treatment of drying and addition of water creating an environment suitable for the growth of particular microorganisms. Food contaminant like *Staphylococcus epidermis, Bacillus cereus* and *Alcaligenes* species were detected in some of the fermented products, as fermented foods in Nagaland are still prepared at the household level using traditional methods. Poor hygienic standards during the preparation of these fermented method also explains the presence of the contaminants in the food product.

Thus, improvement of crude traditional methods by employing modern scientific technologies is the need of hour to upgrade the quality and production of fermented products at commercial scale while keeping intact their unique natural flavour, taste and aroma. Knowledge of microbial diversity and dynamics during spontaneous fermentations is useful, for designing relevant starter cultures that may result in

fermented products with standardized sensory profiles (appearance, aroma, sourness and taste) and fermentation time. Ultimately, these starter cultures may also be used for upgrading this traditional technology to large-scale industrial production and marketing of these fermented food products. The entire process is therefore important, not only from an academic viewpoint, but also for the conservation of indigenous knowledge and technologies through the characterization and preservation of the microflora associated with the traditional fermented food products.

Chapter - 4

Nutritional Analysis of Fermented Food Products

Nutritional factors are widely considered to be critical for human health. Nutrition is the science of food, the nutrients and other substances in it. It deals with their action, interaction and balance in relationship to health and disease. Nutrition is also concerned with socio-economic, cultural and psychological implication of food. Nutritive value refers to the nutrient content of a specific amount of food. Nutrients promote health by making possible the normal operation and maintenance of the body. No matter how different people are in size, appearance, activity, and race or age, all need the same nutrients (Nicoli et al., 1999; Seed et al., 2013). Foods in addition to their nutritional and sensory properties have recently been recognized as acting as protective agents as well and are termed as functional foods which was first introduced in Japan in the mid 1980s and refered to as processed foods containing ingredients that aid specific bodily functions in addition to being nutritious (Arai, 1996).

Modern world is suffering from the problem of shortage of food and billions of people suffer from severe problem of nutrition. Asian, Africans, Latin American countries, North American and Western European countries suffer from malnutrition. The evaluation and documentation of nutrition education is essential to improve impacts and outcomes of efforts. Nutrition education has emerged and evolved along with the science of understanding nutritional needs for health, followed by government recommendations (Müller and Krawinkel, 2005).

Fermented foods also promote human health in ways not directly attributable to the starting food materials. That is, the outcomes of fermentation and the contributions of microbes, in particular, can provide additional properties beyond basic nutrition (Gibson et al., 2006; Mohite, 2013). The health benefits of fermented functional foods are expressed either directly through the interactions of ingested live microorganisms with the host (probiotic effect) or indirectly as the result of the ingestion of microbial metabolites synthesized during fermentation (biogenic effect) (Stanton et al., 2005; Gobbetti et al., 2010; Marco et al., 2017).

Fermentation can be viewed as a biological method of food preservation and enhances taste and flavour of the foods (Motarjemi, 2002). Fermentation also improves protein quality and digestibility, vitamin B content, and microbiological safety and keeping quality (Hotz and Gibson, 2007; Mulaw and Tesfaye, 2017). Foods produced in this way have a reduced risk of contamination by controlling the growth and multiplication of a number of pathogens in foods when enriched in antimicrobial end-products, such as organic acids, ethanol, and bacteriocins. Advantages of fermented foods also include the new and desirable tastes and textures that are completely unlike those present in the starting materials (Marco et al., 2017). Thus, it makes an important contribution to human nutrition and food safety, particularly in developing countries

(Motarjemi, 2002; Renjini, 2014). Fermentation produces acids and alcohol, which in combination is conductive to the formation of esters that impart desirable flavours. Produced carbon dioxide replaces the air and provides anaerobic conditions favourable for the stability of ascorbic acid and the natural colour of vegetables (Lee, 1997).

Fermentation by certain bacteria, yeast and moulds, contain enzymes, which include amylase, proteases, phytases and lipases, modifies the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively. Fermentation also reduces the levels of anti nutrients such as phytic acid and tannins in food leading to increased bioavailability of minerals such as iron, protein and simple sugars (Nout and Motarjemi, 1997; Kumar et al., 2012; Hasan et al., 2014). The lactic acid fermentation enhances protein solubility and the availability of limiting amino acids in some cases by as much as 50%. Tannins are reduced by as much as 50% and oligosaccharides by as much as 90% (Nout and Ngoddy, 1997).

Foods fermented with lactic acid bacteria are considered to have several beneficial physiological effects such as antimicrobial activity enhancing the immune potency (Kullisaar et al., 2002) and to prevent cancer and lower serum cholesterol levels (Kaur et al., 2002).The antioxidant activity of fermented foods improves primarily due to an increase in the amount of phenolic compounds and flavonoids during fermentation, which is the result of a microbial hydrolysis reaction. Moreover, fermentation induces the structural breakdown of plant cell walls, leading to the liberation or synthesis of various antioxidant compounds (Juan and Chou, 2010; Wang et al., 2014). These antioxidant compounds can act as free radical terminators, metal chelators, singlet oxygen quenchers, or hydrogen donors to radicals. The production of protease, amylase and some other enzymes can be influenced by fermentation that may have metal ion chelating activity (Kim et al., 2008; Sarmadi and Ismail, 2010; Hur et al., 2014). While

many health claims have yet to be demonstrated clinically, various scientific and government bodies around the world endorse the 'functional' properties of fermented foods (Gorman, 2011). In the present study attempt was made to investigate into the certain nutritional parameters of five major fermented food products and compare with the constituent raw materials.

Materials and Methods

The nutritional analyses were conducted on following five major fermented products and constituent raw materials:

1. *Axone/Akhuni* prepared from soybean seeds.
2. *Anishi* prepared from *Colocasia* leaves.
3. *Hungrii* prepared from *Brassica* leaves.
4. *Rhujuk*/*Bastanga* prepared from young succulent bamboo shoots.
5. *Tsutuocie* prepared from cucumber.

These foods were selected as they are popularly consumed in the daily diet of almost all the *Naga* tribes in Nagaland. Nutritional assessment was done with an aim to do comparative analysis and to have an idea about the nutritional value addition in the fermented foods. The fermented food products with raw materials are illustrated in **figure 4.1**.

Collection and Preparation of Samples

The fermented food samples were collected from different households from different parts of Nagaland and brought to the laboratory and stored at 4°C till use. The collection of raw materials was done during the particular seasons when they were most abundantly grown. Soybean seeds and cucumber fruits were brought from the local market in Nagaland. *Colocasia* leaves and *Brassica* leaves were collected in the month of August-December. Young succulent bamboo shoots were collected in the month of June-

July. For the assessment of nutritional value, all the samples were first oven dried at 60ºC and were grounded to a fine powder. The finely powdered samples were kept separately in an airtight container at 4ºC until the time of use.

Methodology

Proximate Analysis

Moisture content: Moisture content was estimated by taking 5 g of sample in a pre-weighed dish plate and placed in the oven for ~16 h at 70±1°C till a constant weight was achieved. After drying, samples were weighed again and the moisture content was determined by using the formula:

$$\text{Moisture content (\%)} = \frac{\text{Loss of weight}}{\text{Weight of the sample}} \times 100$$

Estimation of protein: Protein estimation was done using the colorimetric method of Lowry et al. (1951). One gram of oven dried sample was grounded using 20 ml of 0.1 M phosphate buffer (*p*H - 7.0) (**Appendix-I**) and centrifuged at 1000 rpm for 10 min followed by filtration and filtrate was used for the analysis. To 1 ml of extract 5 ml of Lowry's solution (**Appendix-I**) was added. The mixture was incubated at room temperature for 10 min followed by added 0.5 ml of 1N Folin-Ciocalteau reagent (**Appendix-I**) and incubated in dark for 20 min. The absorbance at 660 nm was measured and standard curve was prepared with 'Bovine Serum Albumin' (BSA).

Estimation of reducing sugar: Reducing sugar was estimated using 3, 5-dinitrosalicylic acid (DNSA) reagent (**Appendix-I**) (Miller, 1959). One gram of oven dried sample was extracted with 80% alcohol. To 1 ml of extract 1 ml of DNS reagent was added and mixture was kept in boiling water bath for 5 min followed by cooling to room temperature with 10 ml distilled water. The absorbance was measured at 540 nm and glucose was taken as the standard.

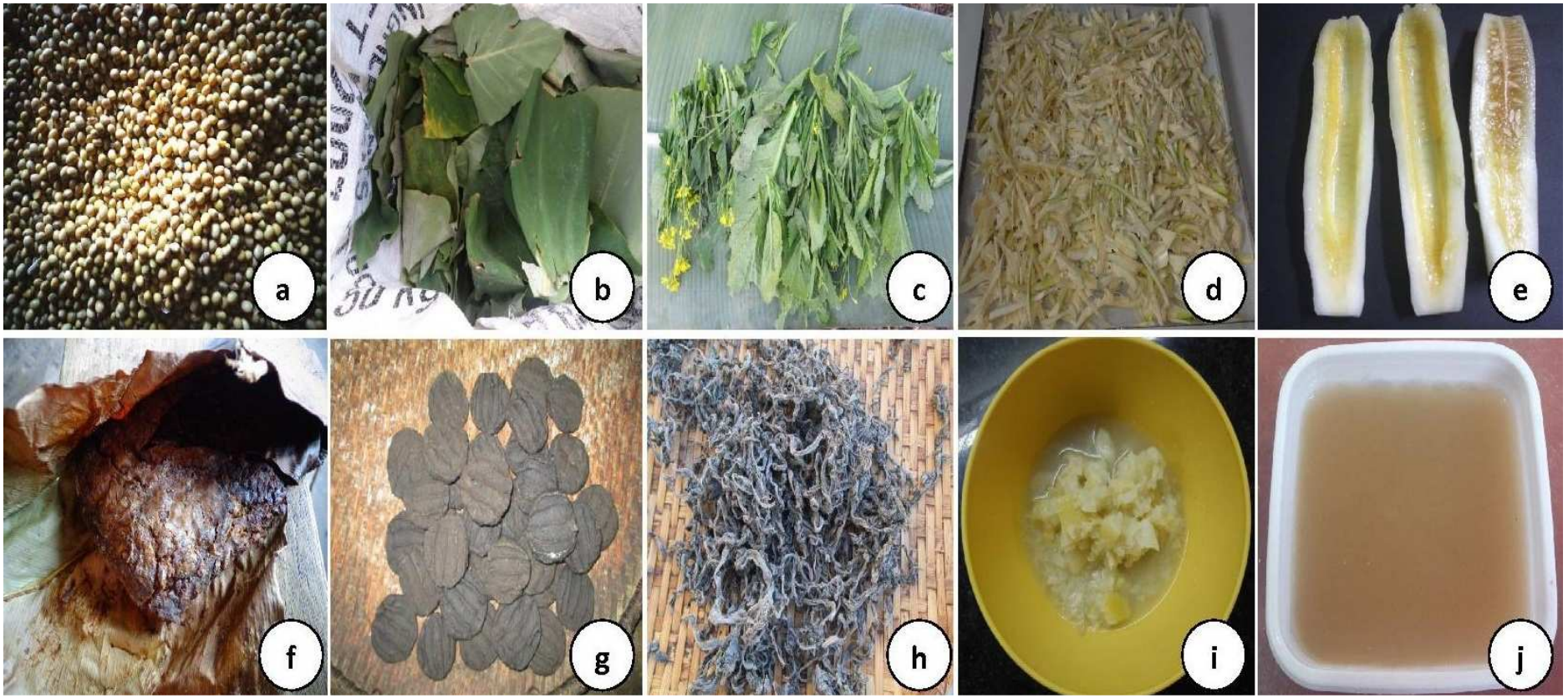


Figure - 4.1

Figure 4.1: Principle constituents and the final fermented products. a- Soybean seeds, b. *Axone/akhuni*, c. *Colocasia* leaves, d. *Anishi*, e. *Brassica* leaves, f. *Hungrii*, g. Young succulent bamboo shoots, h. *Rhujuk/bastanga*, i. Cucumber slices, j. *Tsutuocie*

Estimation of crude fibre: Crude fibre was determined following Maynard (1970) with modification. One gram of dried samples was boiled with 200 ml of 0.25N sulphuric acid (H_2SO_4) for 30 min. It was then filtered with No. 1 Whatman filter paper. The filtrate was then boiled with 200 ml of 0.313N NaOH solution for 30 min followed by filtration and washed subsequently with 25 ml of boiling 1.25% H_2SO_4 and thrice with 50 ml distilled water and 25 ml of alcohol. The residue was removed and transferred to pre-weighed ashing dish (W_1g). The filtrate was then dried for 2 h at 130±2ºC and then cooled. The ashing dish was cooled and weighed (W_2g). It was ignited for 30 min at 600°C. After cooling in desiccator, it was again reweighed (W_3 g). The crude fibre content was determined using the formula:

$$\text{Crude fibre (g/100g)} = \frac{\text{Loss in weight on ignition } (W_2\text{-}W_1) - (W_3\text{-}W_1)}{\text{Original weight of sample}} \times 100$$

***p*H of food products:** Five grams of sample was blended with 10 ml of distilled water in a homogeniser and the *p*H of the slurry was determined directly using a digital *p*H meter.

Total phenolic and flavonoid content

Preparation of methanol extract: One gram of dried sample was ground and extracted in 10 ml of 80% (v/v) methanol by shaking for 24 h at room temperature. The extraction procedure was repeated until the extraction solvent became colourless. The extract was then filtered over with Whatman No. 4 filter paper. The filtered obtained was directly used for antioxidant analysis.

Estimation of total phenolic content (TPC): Total phenol content was determined following Folin-Ciocalteau method (Singleton and Rossi, 1965). About 0.1 ml extract was added to 1 ml Folin-Ciocalteau reagent and 0.9 ml of distilled water and allowed to stand for 5 min followed by mixing of 2 ml of saturated sodium carbonate (75 gL^{-1}) and 2 ml of water. The absorbance was measured at 765 nm after incubating at 30°C for 1 h

with intermittent shaking. Gallic acid was used for making the standard graph and expressed as mg Gallic acid equivalents (GAE) / g of extract.

Estimation of total flavonoid content (TFC): Total flavonoid content was determined following technique of Sahreen et al. (2010) with slight modification. To 0.3 ml of extract, 3.4 ml of methanol (30%, v/v), 0.15 ml of 0.5M sodium nitrite and 0.15 ml of aluminium chloride (0.3 M) were added. The mixture was then allowed to stand for 5 min and then added 1 ml of 1 M NaOH. The absorbance was measured at 510 nm and standard curve was prepared using Quercetin and expressed as mg Quercetin equivalents (QE) / g of extract.

Antioxidant Activity

DPPH radical scavenging assay: The scavenging activity of stable 2, 2-Diphenyl-1-picrylhydazyl (DPPH) free radical was determined following Aoshima et al. (2004) with modification. Equal volumes of methanolic solutions of DPPH (100 μM) and crude extract containing different concentrations (10-200 μg/mL) were mixed together. The reaction mixture was shaken well and allowed to stand at room temperature for 30 min before reading the absorbance at 517 nm in spectrophotometer (Multiskan Go, Thermo Scientific). Standard curve was calculated using Trolox and inhibition percentage was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Statistical Analysis

The experiments were done in triplicate (n=3) and expressed as mean ± standard deviation. The results were processed using statistical software Microsoft Excel and Origin-Pro 8.

Results

Proximate analysis

For all the selected fermented food products, investigation was under taken on estimation of moisture content, crude fibre, protein, reducing sugar and *p*H. Efforts were made for comparative assessment of different nutrition parameters between the constituent raw materials and the final processed fermented product. The results are shown in respective graphs showing nutritional comparison between the fermented food products and its raw materials (Table 4.1).

Moisture content: The moisture content of the fermented food products showed significant variations ranging from 3.8 to 92%. The maximum moisture content was recorded as 90 and 92% in *rhujuk/bastanga* and *tsutuocie* respectively, which had no significant difference from the moisture content in its raw material, cucumber fruit and young succulent bamboo shoots (82% and 92%). Moisture content was significantly lower in *anishi* and *hungrii* having 3.8 and 5.2% respectively, than its constituent raw materials i.e., *Colocasia* leaves and *Brassica* leaves which were 60.6 and 40% respectively. However, the moisture content in *axone/akhuni* which was found to be 50% was significantly higher than its raw material soybean seeds having 11.2% (Table 4.1).

Protein content: Protein content of *axone/akhuni, anishi* and *hungrii* were 42.1, 34.19 and 34.07 g per 100 g respectively and it was found to increase as compared to its raw material i.e., soybean seeds (41.8), *Colocasia* leaves (20.64) and *Brassica* leaves (23.34). However, protein content of *rhujuk/bastanga* (30.89) and *tsutuocie* (3.2) were found to decrease compared to its constituent raw materials i.e., succulent bamboo shoots (33.09) and cucumber fruit (6.7). Highest level of protein content was thus reported in *axone/akhuni*, followed by *anishi, hungrii* and *rhujuk/bastanga*. The lowest level of protein content was found in *tsutuocie*.

Table 4.1: Proximate composition of the fermented products in comparison with its raw materials

Parameters	**Axone**		***Anishi***		***Hungrii***		***Rhujuk/ Bastanga***		***Tsutuocie***	
	Raw material (soybean)	**Product**	**Raw material (*Colocasia* leaves)**	**Product**	**Raw material (Brassica leaves)**	**Product**	**Raw material (Bamboo shoot)**	**Product**	**Raw material (Cucumber)**	**Product**
Moisture (%)	**11.2** (0.02)	**50.0** (0.01)	**60.0** (0.1)	**3.8** (0.06)	**40.0** (0.04)	**5.2** (0.03)	**82.0** (0.1)	**90.0** (0.03)	**90.0** (0.04)	**92.0** (0.04)
***p*H**	**6.8** (0.003)	**8.0** (0.001)	**6.2** (0.04)	**5.8** 0.02)	**6.2** (0.05)	**5.2** (0.01)	**6.2** (0.03)	**4.7** (0.07)	**6.1** (0.09)	**8.2** (0.006)
Protein (g/100g)	**41.8** (0.004)	**42.1** (0.03)	**20.64** (0.03)	**34.19** (0.005)	**23.34** (0.1)	**34.07** (0.1)	**33.07** (0.002)	**30.89** (0.1)	**6.7** (0.04)	**3.2** (0.04)
Reducing sugars (%)	**27.6** (0.013)	**29.7** (0.01)	**54.7** (0.1)	**29.6** (0.04)	**32.1** (0.004)	**34.5** (0.04)	**52.1** (0.05)	**29.6** (0.03)	**17.5** (0.007)	**22.5** (0.06)
Crude fibre (g/100g)	**1.04** (0.03)	**1.61** (0.01)	**10.52** (0.1)	**12.26** (0.02)	**2.88** (0.03)	**1.019** (0.06)	**0.17** (0.04)	**0.27** (0.009)	**0.128** (0.04)	**0.05** (0.04)

Data represent mean of three replicates (±Standard deviation).

Reducing sugar: It was found that reducing sugar content decreased considerably in *anishi* (29.6%) and *rhujuk/bastanga* (29.8%) compared to its counterparts i.e., *Colocasia* leaves and young succulent bamboo shoots (54.7 and 52.1% respectively). However, there was increase in the level of reducing sugars in *axone/akhuni* (29.7%), *hungrii* (34.5%) and *tsutuocie* (22.5%) as compared to its raw material, soybean seeds, *Brassica* leaves and cucumber fruit which were found to be 27.6, 32.1 and 17.5% respectively. The maximum level of reducing sugar was found in *hungrii*, followed by *axone/akhuni*, *anishi* and *rhujuk/bastanga.* The lowest level of reducing sugar was found in *tsutuocie* (Table 4.1).

Crude fibre content: Crude fibre was low in *tsutuocie* (0.05g/100g) and *hungrii* (1.019g/100g)) as compared to cucumber fruit (0.128g/100g) and *Brassica* leaves (2.88g/100g). There was however, increase in the level of crude fiber in *axone/akhuni* (1.61g/100g), *anishi* (12.26g/100g) and *rhujuk/bastanga* (0.27g/100g) in comparison to its raw material, soybean seeds, *Colocasia* leaves and young succulent bamboo shoots (1.04, 10.5 and 0.17g/100g respectively). The maximum content of crude fiber was found in *anishi*, followed by *axone/akhuni, hungrii* and *rhujuk/bastanga.* The lowest level of crude fiber was found in *tsutuocie.*

***p*H:** The *p*H levels of *anishi, hungrii* and *rhujuk/bastanga* were observed to decrease from that of its raw material (6.2, 6.2 and 6.2), to be acidic with *p*H value of 5.8, 5.2 and 4.7 respectively. While, *axone/akhuni* and *tsutuocie* were found to have increased *p*H value of 8 and 8.2 respectively, from that of its raw material having *p*H level of 6.8 and 6.1 respectively.

Total phenol and flavonoid content

In general, fermented foods are known for high flavonoid and TPC and due to higher content of these, fermented foods are considered to be value food for the ethnic

tribes. In the present study effort was made to quantify the total phenol content and flavonoid content in the selected fermented food and compare with constituent raw materials. Results of present study shows differential TPC and flavonoid content in different fermented food products. Further, when compared with its raw materials, it was found that in three products there was increase in TPC and in four products, there was increase in flavonoid content compared to the raw materials. Two products exhibited decrease in content of TPC and one product in flavonoid content. Comparison of total phenolic and flavonoid content of the fermented products and its raw materials is given in figure 4.2, 4.3, 4.4, 4.5 and 4.6. The data revealed that the amount of total phenolic content of *axone/akhuni* (0.86 mg GAE/g), *anishi* (1.44 mg GAE/g) and *rhujuk/bastanga* (2.44 mg GAE/g) were significantly higher than in the raw materials soybean seeds (0.2 mg GAE/g), *Colocasia* leaves (0.88 mg GAE/g) and young succulent bamboo shoots (1.52 mg GAE/g) (Figure 4.2, 4.3 and 4.4). However, *hungrii* (1.66 mg GAE/g) and *tsutuocie* (0.22mg GAE/g) had relatively lower levels of phenolics as compared to *Brassica* leaves (2.72 mg GAE/g) and cucumber fruit (0.4 mg GAE/g) (Figure 4.5 and 4.6). Out of the five fermented food products it was observed that *rhujuk/bastanga* had the highest level of phenolic content, followed by *hungrii, anishi* and *axone/akhuni.* The lowest level of phenolic content was observed in *tsutuocie.*

Total flavonoid content in *axone/akhuni* (0.64 mg QE/g), *anishi* (2.06 mg QE/g), *rhujuk/bastanga* (0.62 mg QE/g) and *tsutuocie* (0.12 mg QE/g) increased significantly than in soybean seeds (0.46 mg QE/g), *Colocasia* leaves (0.66 mg QE/g), young succulent bamboo shoots (0.36 mg QE/g) and cucumber (0.028 mg QE/g) (Figure 4.2, 4.3, 4.4 and 4.6). *Hungrii* (0.76 mg QE/g) had lower level of flavonoid as compared to brassica leaves (1.08 mg QE/g) (Figure 4.5). Out of the five fermented food products it was observed that *anishi* had the highest level of flavonoid content, followed by *hungrii*,

axone/akhuni and *rhujuk/bastanga.* The lowest level of flavonoid content was observed in *tsutuocie.*

Antioxidant activity

Fermented foods are known to be rich in pharmaceutical compounds due to higher antioxidant activity, presence of higher TPC, phenols etc. Comparison of total antioxidant activity of the fermented products and its raw materials is given in table 4.2 and figure 4.7. Free radical scavenging activity for DPPH radical was expressed as IC50 value (the concentration required to scavenge 50% of DPPH). By increasing the plant extract concentration there was a corresponding continuous increase in scavenging activity. The free radical scavenging activity of *axone/akhuni* (98.79 µg/ml), *anishi* (60.2 µg/ml) and *rhujuk/bastanga* (92.85 µg/ml) was higher than in soybean seeds (186.75 µg/ml), colocasia leaves (100.9 µg/ml) and young succulent bamboo shoots (112.3 µg/ml). However, the free radical scavenging activity of *hungrii* (73.3 µg/ml) and *tsutuocie* (219.3 µg/ml) were lower compared to brassica leaves (65.7 µg/ml) and cucumber fruit (120 µg/ml). Maximum antioxidant activity was recorded in *anishi*, followed by *hungrii*, *rhujuk/bastanga* and *axone/akhuni*. Lowest antioxidant activity was recorded in *tsutuocie* (Table 4.2 and Figure 4.7).

The results obtained in the present study revealed that the fermented food products, *axone/akhuni, anishi, hungrii* and *rhujuk/bastanga* are highly nutritious and are good source of anitioxidants, thus they can be consumed not only as food but also as curative. However, *tsutuocie* was found to be less nutritious but can still be consumed as a condiment to add flavour and aroma to otherwise bland food.

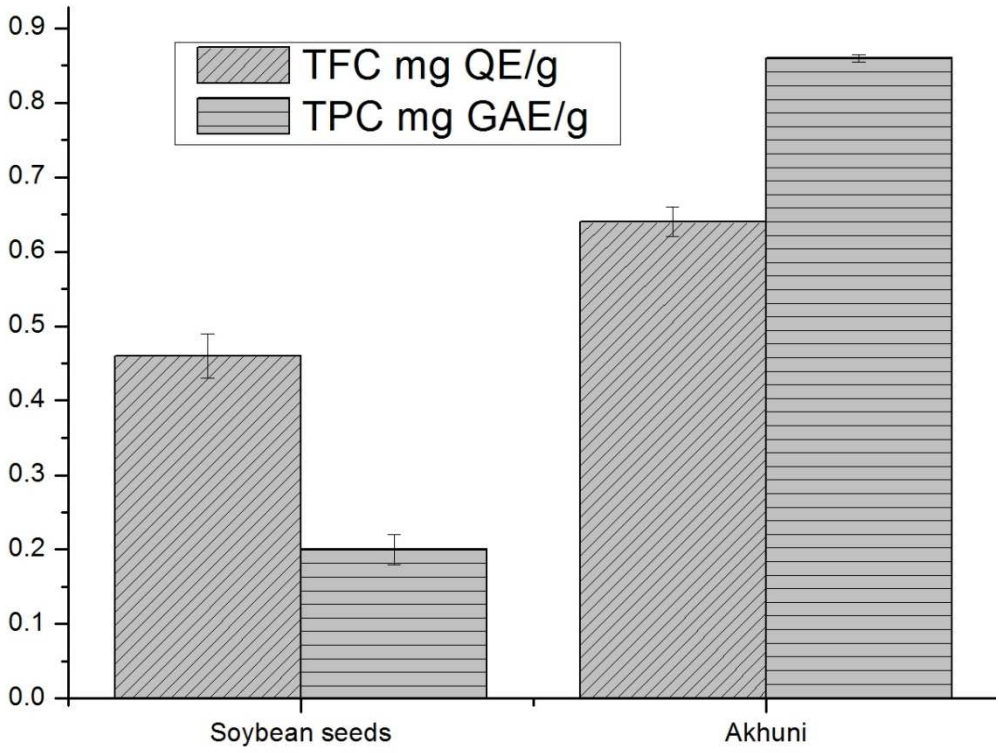


Figure - 4.2

Figure 4.2: Total phenol and flavonoid contents in soybean seeds and its fermented product (*Axone/Akhuni*)

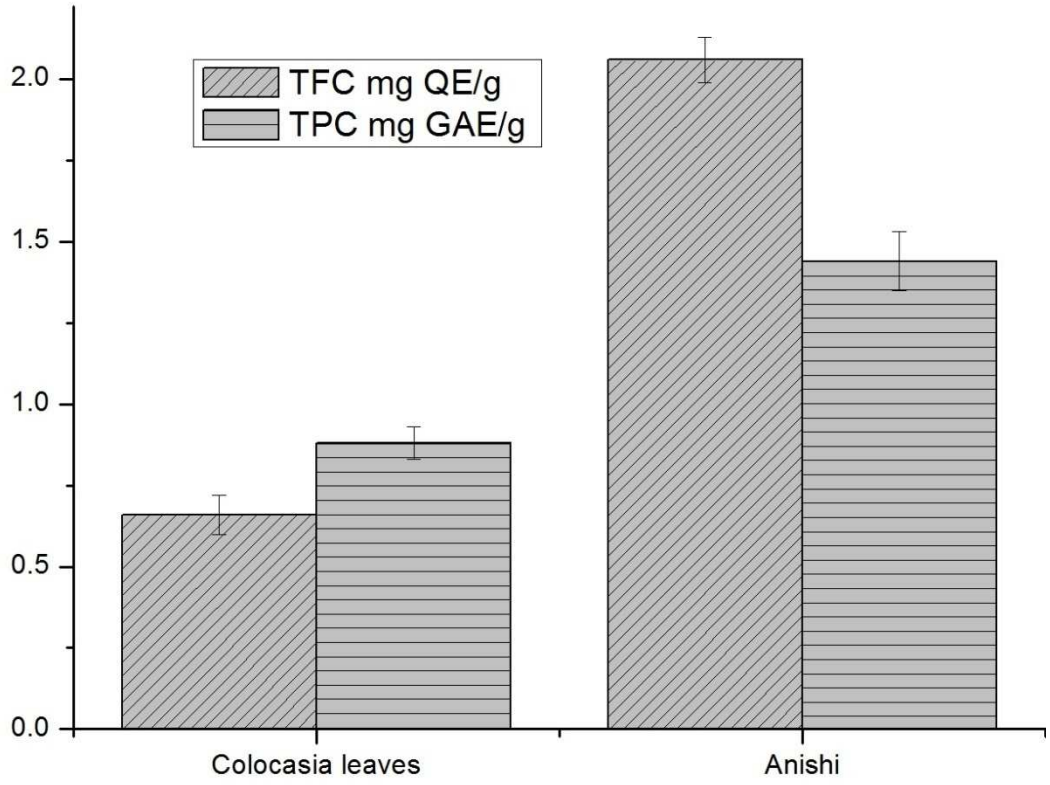


Figure - 4.3

Figure 4.3: Total phenol and flavonoid contents in *Colocasia* leaves and its fermented product (*Anishi*)

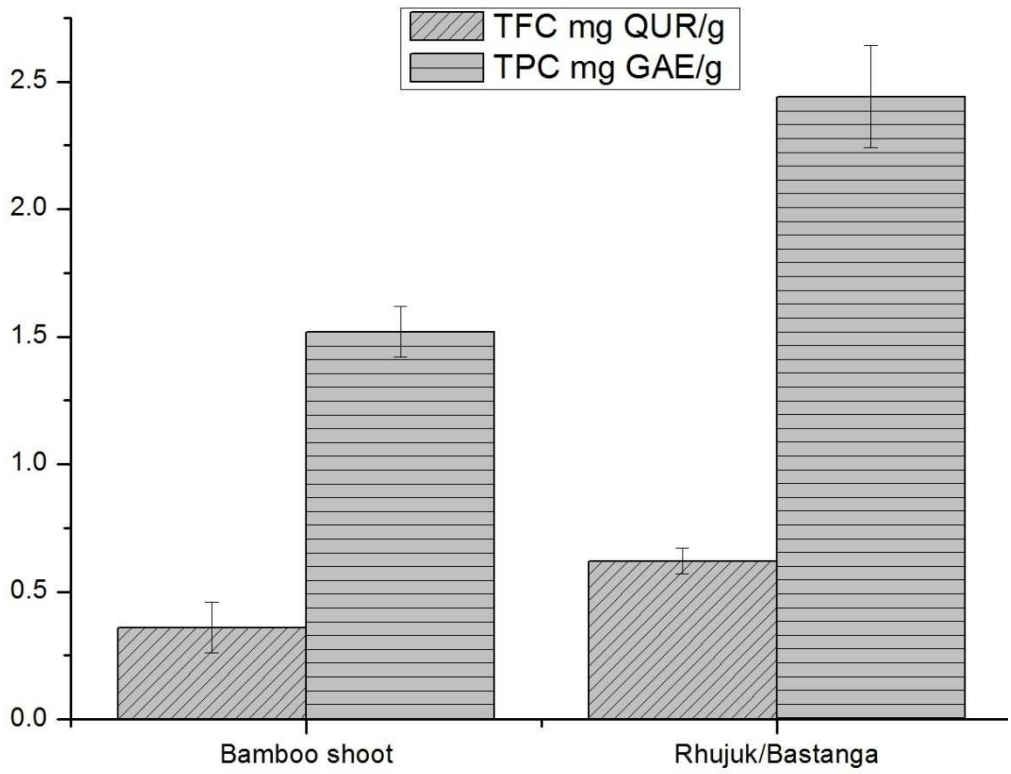


Figure - 4.4

Figure 4.4: Total phenol and flavonoid contents in bamboo shoots and its fermented product (*Rhujuk/Bastanga*)

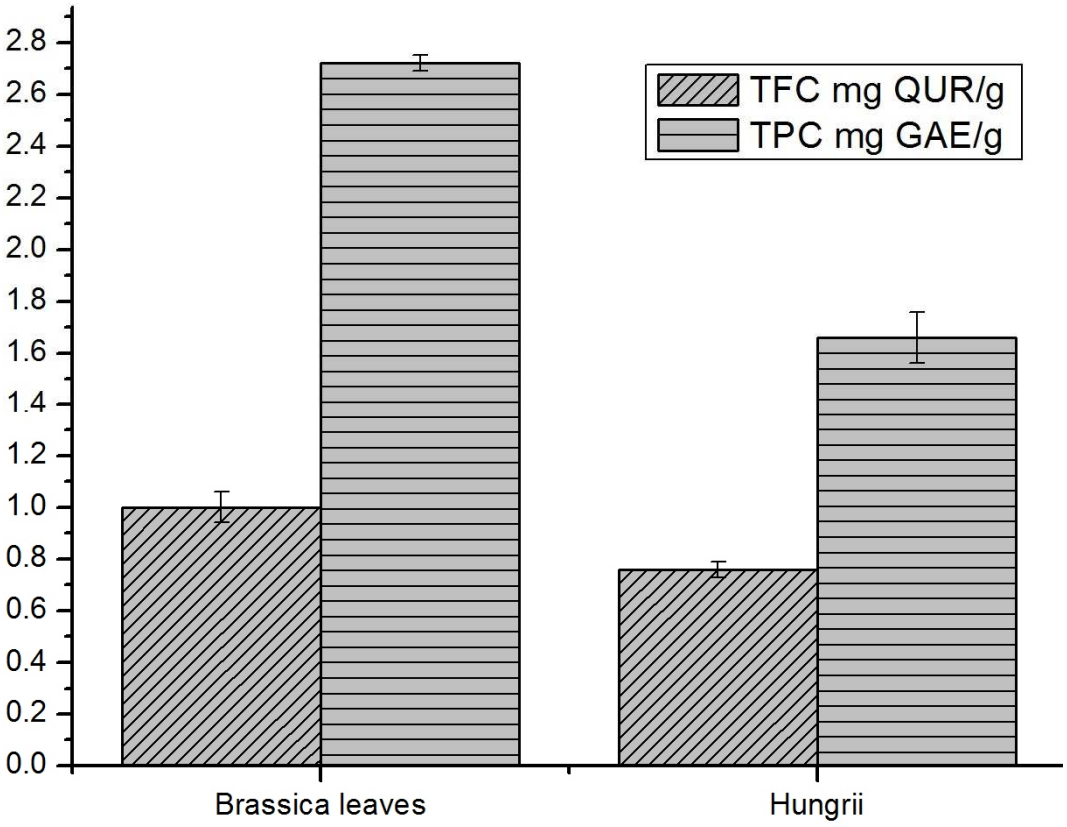


Figure - 4.5

Figure 4.5: Total phenol and flavonoid contents in brassica leaves and its fermented product (*Hungrii*)

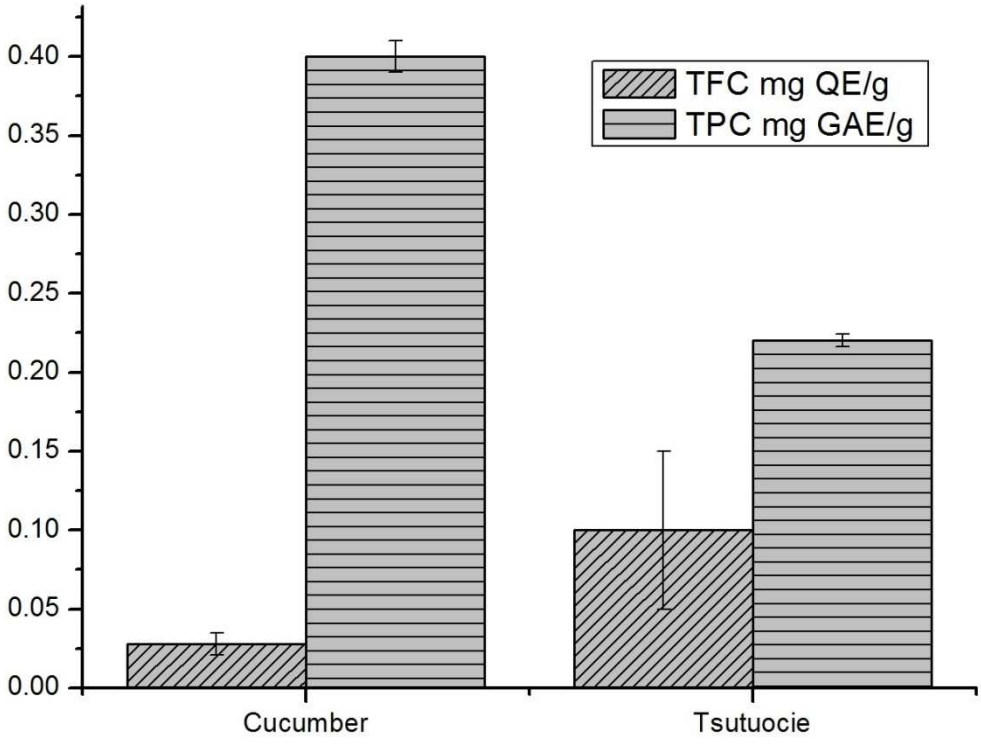


Figure - 4.6

Figure 4.6: Total phenol and flavonoid contents in cucumber and its fermented product (*Tsutuocie*)

Table 4.2: DPPH IC50 values of fermented products in comparison to its raw materials

Raw materials	DPPH IC50 (µg/ml)	Fermented product	DPPH IC50 (µg/ml)
Soybean seeds	186.75	*Axone/akhuni*	98.79
Colocasia leaves	100.9	*Anishi*	60.2
Brassica leaves	65.7	*Hungrii*	73.3
Bamboo shoot	112.3	*Rhujuk/Bastanga*	92.85
Cucumber	120.8	*Tsutuocie*	219.32
Trolox			240.50

Data represent mean of three replicates.

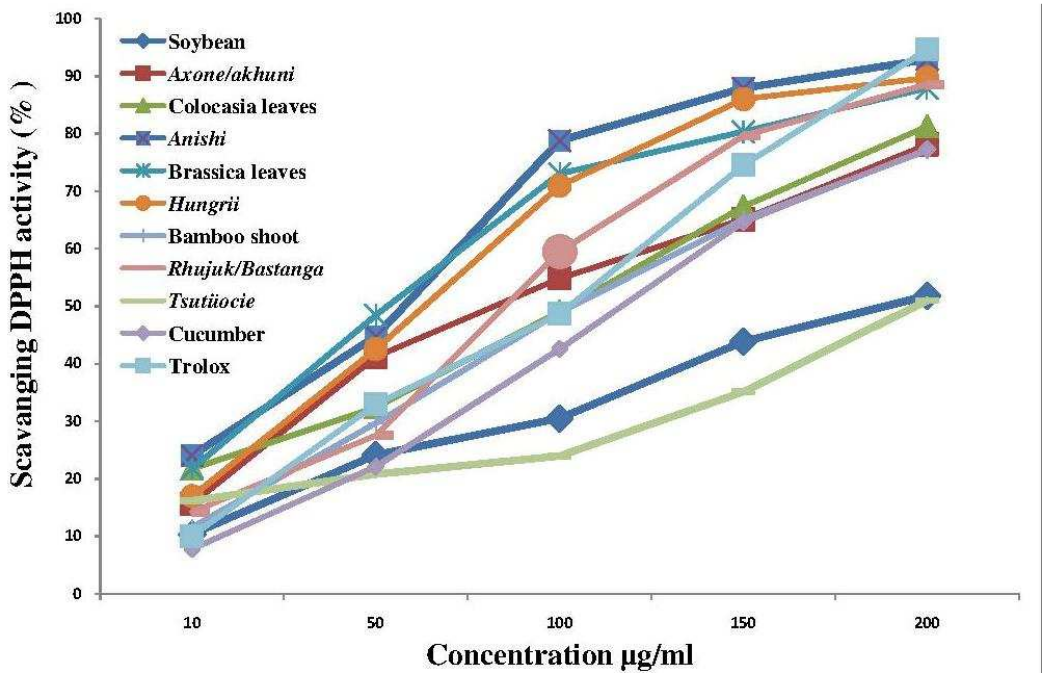


Figure - 4.7

Figure 4.7: DPPH IC50 values of fermented products in comparison to its raw materials

Discussion

It is a well known fact that during fermentation process there are many biological changes including involvement of microbes, biochemical reactions which leads to changing the biochemical constituents of a particular raw material. These changes alter the texture, aroma of fermented food and add nutritional values to the fermented foods. In Nagaland, even though fermented foods form an integral part of daily *Naga* diet, its nutritional attributes are still not documented and studied. This present investigation thus puts some light on the proximate composition as well as the antioxidant content of some major fermented foods of Nagaland so as to popularise these products as nutritional support to the region for health improvement.

Legumes are the main source of nutrients for traditional complementary foods in developing countries. Legumes such as soybean are one of the richest and cheapest sources of plant protein that can be good substitute for animal products (Tufa et al., 2016). Legumes are also rich in micronutrients; however, the availability of those nutrients is usually low due to the presence of antinutritional factors such as phytic acid and tannins. Fermentation and soaking are simple traditional processing treatments that decrease the level of antinutrients in legumes and increase the nutrients content of the diet (Iwuoha and Eke, 1996). The qualitative and quantitative composition of soybean components is dramatically changed by physical and enzymatic processes during the preparation of soy-based foods (Kwon et al., 2010). The large protein, lipid, and carbohydrate molecules in raw soybean are broken down by enzymatic hydrolysis during fermentation to small molecules such as peptides, amino acids, fatty acids, and sugars, which are responsible for the unique sensory and functional properties of the final products (Fabiyi, 2006). The antioxidant activity of fermented soybean has been reported due to presence of polyphenols and phytochemicals in soya fermented foods (Yang et al.,

2000; Amadou et al., 2009; Chonkeeree et al., 2013). Besides antioxidant, soybean is also known for rich isoflavones and phenolic compounds and increase further during fermentation (Samruan et al., 2012; Moktan et al., 2008). Soya isoflavones like genistein and daidzein have been reported to have inhibitory effect on the breakage of DNA induced by hydrogen peroxide (Kim et al., 2008). The health benefits of fermented soy foods have been attributed to the antioxidant capacity of particular compounds structurally modified or released after bacterial hydrolysis (Hubert et al., 2008).

Preservation of perishable and seasonal vegetables in absence of refrigeration and freezing is an age old practice. These preserved fermented vegetables are consumed during the long monsoon season, when fresh vegetables are not available (Tamang and Tamang, 2009). Fermented fruits and vegetables contain toxins and antinutritional compounds, which can be removed or detoxified by the action of microorganisms during fermentation process (Smith and Eyzaguirre, 2007). The fermentation of vegetables is hard to control, due to its variations in shape, size and type of naturally occurring microorganisms, leading to variability in their nutrient content (Buckenhuskes, 1993). Bioreaction in vegetable fermentation normally involve minor changes in both nutrients and other physiochemical properties of vegetables. Changes in vegetable nutrients include increasing free amino acids, improvement in protein digestibility and development of desirable flavour and colours (Perricone et al., 2017). Phenolic compounds are components in vegetables that are directly related to food flavour, astringency and colour when degraded by microorganisms. The presence of phenolic compounds in the diet may benefit host health because of their chemo preventive activities against carcinogenesis and mutagenesis (Rodriguez et al., 2009). In Nagaland, agriculture being the main occupation, preservation of perishable crops has been practiced since time immemorial. It not only contributes to the dietary intake but also

improves safety, quality and availability of food and generates income to the rural people. Sun-drying and fermentation are the two important traditional techniques undertaken for the processing of vegetables. The vegetable based fermented food products taken up for this present investigation *viz. anishi, hungrii, rhujuk/bastangaa* and *tsutuocie* showed variations in their proximate composition. Fermented products like *anishi, hungrii* and *rhujuk/bastanga* were found to be good source of protein; *anishi* and *hungrii* were found to be good source of crude fiber and *anishi, hungrii* and *rhujuk/bastanga* were found to contain fairly good amount of phenol and flavonoid rendering them to have antioxidant ability. The fermented product *tsutuocie* was found to be less nutritious than the other products.

Proximate composition

Moisture content is the quantity of water contained in a material. The amount of moisture (water) in the food has an important influence on the calorific value and defines the shelf life. Water is an essential compound of many foods (Ellaiah et al., 2002). Moisture content of *axone/akhuni* increased considerably from 11.2% to that of 50%, when compared to its raw material, due to the addition of water during cooking and washing of the cotyledon (Omadara and Olowomofe, 2015). Previous studies also showed similar results in other soybean based fermented products like *thua nao* of Thailand having moisture content of 57.22-64.78% (Chukeatirote, 2015); soy-*iru* of Africa having moisture content of 59% (Omadara and Olowomofe, 2015); *kinema* of Nepal having moisture content of 62% and in raw soybean having 10.8% respectively (Sarkar et al., 1994; Tamang, 2010); *hawaijar* of Manipur having 60% of moisture content (Premarani and Chhetry, 2011; Keishing and Banu, 2013); *tungrymbai* of Meghalaya having 60% of moisture content (Agrahar-Murugkar and Subbulakshmi, 2006) and *bekang* of Mizoram having 63.5% of moisture content (Tamang et al., 2012).

Moisture content of *anishi* (3.8%) and *hungrii* (5.2%) were low in comparison to its raw material, as they were baked or dried after fermentation, other vegetable based fermented products also showed low moisture content such as *gundruk* and *sinki* of Nepal having 15% and 22.8% respectively (Tamang et al., 2012). While, Moisture content of *rhujuk/bastanga* (90%) and *tsutüocie* (92%) had high moisture content this may be due to addition of water during its preparation (Tamang et al., 2009). Bamboo shoot based fermented products like *mesu* of Nepal, *soibum* of Manipur and *ekung* of Arunachal Pradesh also showed high moisture content of 89.9%, 92.0 % and 94.7% respectively (Tamang et al., 2012; Sonar et al., 2015).

Protein is used for building and repairing of body tissues, regulation of body processes and formation of enzymes and hormones. Proteins also aid in the formation of antibodies that enable the body to fight infection. Proteins serve as a major energy supplier (Brosnan, 2003). Protein deficiency is an important cause since protein is essential for both growth and maintenance of muscle mass (Elango and Laviano., 2017). During fermentation protein content of food as a whole changes, as they are either hydrolyzed into their component amino acids, some of which gets absorbed and converted to microbial protein, whereas some are used up in secondary metabolism (Paredes-Lopez et al., 1988). In the present study protein content of *axone/akhuni* was 42.1 g/100g, which was found to increase just slightly from its raw material soybean seeds having 41.8 g/100g respectively. Increases in protein content during fermentation, may be due to the proteolytic activities of enzymes produced by microorganisms which increases the bioavailability of amino acids (Sanjukta and Rai, 2016). Several studies indicated the increase in protein content of soybean based fermented products as compared to its raw material such as in *hawaijar*, the protein content was 43.8%, which increased from soybean having 35% respectively (Keishing and Banu, 2013); *tungrymbai*

contained 45.9 g/100g of protein as compared to 43.2g/100g in the unfermented counterpart (Agrahar-Murugkar and Subbulakshmi, 2006); protein content in *kinema* was reported to be 47.7g/100g, which increased slightly from raw soybean having 47.1g/100g of protein respectively (Sarkar et al., 1994; Tamang et al., 2012); soy-*iru* contained 45.53% of protein as compared to the unfermented soybean which contained 43.34% of protein respectively (Omodara and Olowomofe, 2015); *Chungkookjang*, a popular fermented soybean paste in Korea, has been considered to be more healthful than soybeans, which increased from 37.2% to 41.3% respectively (Kwak et al., 2007); *thua nao* contained 38.94-42.06% of protein (Dajanta et al., 2012).

In the present study the protein content in *anishi*, prepared from *Colocasia* leaves and *hungrii*, prepared from *Brassica* leaves were found to be 34.19 g/100g and 34.07 g/100g which significantly increased from its raw material having 20.64 g/100g and 23.34 g/100g respectively. The higher percentage of protein in the fermented product may be due to reduction of carbohydrate content of the unfermented samples (Oguntoyinbo et al., 2016). The protein content in other leaf based fermented product like *gundruk*, prepared from mustard, *rayo-sag* (local brassica leaves) and cauliflower leaves was reported to be 37.4% respectively. Another product *goyang*, prepared from leaves of local brassica species of Nepal was reported to contain 35.9% of protein respectively (Tamang, 2010).

Bamboo shoots are known as “wild or forest vegetable” and are consumed either in their fresh form or dried, fermented or pickled and canned (Choudhury et al., 2011; Sonar et al., 2015). The protein content of *rhujuk/bastanga*, prepared from young succulent bamboo shoots was found to contain 30.89g/100g, which decreased from its raw material having 33.09g/100g of protein respectively. The reduction in protein content may be due to the denaturation of protein during fermentation (Bajwa et al., 2016). A

similar result was reported in *khorisa*, a bamboo shoot based fermented product of Assam, where the protein content was lower than its raw material (3.78% to 2.40%) (Chakrabarty et al., 2014). Choudhurt et al. (2011) studied on the nutritional value of bamboo shoot based fermented food products and observed decrease in the protein content in the fermented product compared to its raw material (3.108 g/100g to 2.170 g/100g). However, Agrahar-Murugkar and Subbulakshmi (2006), found enhancement of protein content in *lungsiej*, a fermented bamboo shoot product of Meghalaya from 3.9 g/100g to 8.5 g/100g respectively. Tamang (2010) reported the protein content in the various bamboo shoot based fermented products of the Himalayan regions *viz. ekung, eup, hirring, mesu, soibum* and *soidon* to be 30.1%, 33.6%, 33.0%, 27.0%, 36.3% and 37.2% respectively. Sonar et al. (2015) also reported on the protein content of fermented bamboo shoot products of North East, India viz. *eup, soibum, hecche, hirring, soidon* and *ekung* to be 19.53%, 23.61%, 27.55%, 25.57%, 20.65% and 24.62% respectively and the presence of 27.8% of protein in fresh bamboo shoots.

In the present study the protein content in *tsutuocie*, prepared from ripened cucumber fruits were low compared to its raw material from 6.7g /100g to 3.2g /100g respectively. During its preparation salt is not added so it differs from the pickled cucumber popularly consumed and produced around the world. *Khalpi*, is also a non-salted fermented cucumber based product of Nepal (Tamang, 2010) but its processing method completely differs from that of *tsutuocie*. Tamang (2010) reported the protein content in *khalpi*, to be 12.3% respectively.

Reducing sugars like glucose plays a very important role during fermentation as the microorganisms utilize them to undergo fermentation (Singh et al., 2011). Reducing sugars are also responsible for the change in the colour and taste of the food as the free aldehyde or ketone group which reacts chemically by donating an electron to another

molecule and cause alteration. In the present study the reducing sugar in *axone/akhuni* increased after fermentation from 27.6 to 29.7% respectively. The increase may be due to increase in the activity of native or microbial amylases which hydrolyses starch to sugars (Senthilkumar, et al., 2012). Similar report was also seen in *hawaijar*, where reducing sugars increased as compared to soybean from 1.10 to 3.1% respectively (Keishing and Banu, 2013). Omafuvbe et al. (2000) reported the content of reducing sugars in soy-*daddawa* to be 5.06 mg/g. Dajanta et al. (2012) also reported the reducing sugars in *thua nao* to be in the range of 2.70-7.74%.

The reducing sugars in *hungrii* and *tsutuocie* were also found to increase as compared to its raw material. However, the reducing sugars in *anishi* and *rhujuk/bastanga* decreased as compared to its counterpart. The reduction in the reducing sugar content of fermented product could be as a result of the utilization of some of the sugars by fermenting organisms for growth and metabolic activities (Nongdam and Tikendra, 2014). Singh et al. (2011) reported a sharp consistent decrease in the level of reducing sugars in two varieties of *soibum*, from 1.47 to 0.62 g/100g and 3.16 to 1.2 g/100g respectively.

Studies have indicated that components of plants such as dietary fibre have beneficial effects in lowering blood cholesterol levels aside from the decreased intake of saturated fat and cholesterol that occurs with high intakes of plant foods (Ekumankama, 2008). Fibre cleanses the digestive tract, by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Finally fiber binds to cancer-causing chemicals, keeping them away from the cells lining the colon, providing yet another line of protection from colon cancer (Essiett and Ukpong, 2014). In the present study the crude fiber content in *axone/akhuni* was found to be 1.605g/100g, which increased from soybean seeds having 1.035g/100g. Keishing and Banu (2013) also

reported the increase in crude fiber from 3.51% to 5.56% in *hawaijar* as compared to soybean. However, Omadara and Olowomofe (2015) reported decrease in the level of crude fiber in soy-*iru* from 5.63% to 4.54% in the unfermented soybean. The crude fibre content in *sufu* was reported to be in the range of 0.2-1.5% (Cheng and Han, 2013); in *thua nao* it was reported to be in the range of 2.70-7.74% (Dajanta et al., 2012) and in *tungrymbai* it was reported to be 12.8g/100g (Agrahar-Murugkar and Subbulakshmi, 2006).

The crude fibre content in *rhujuk/bastanga*, increased from its raw material i.e. from 0.172 to 0.265 g/100g respectively. The crude fibre in other bamboo shoot based fermented products had wide variations in comparison with the results in the present study, which may be due to the different bamboo species used and also on their conditions of growth (Chandramouli and Viswanath, 2015). Nongdam and Tikendra (2014) studied the nutrient composition in different bamboo species such as *Dendrocalamus strictus, Dendrocalamus asper, Bambusa vulgaris* and *Bambusa nutans,* and the crude fibre content in its raw form was reported as 0.98 g/100g, 0.70 g/100g, 0.70 g/100g and 0.76 g/100g respectively. They also reported the fibre content in one of the fermented bamboo shoot species, which was found to be 0.21 g/100g. Other bamboo shoot based fermented products in North East, India was studied for its nutritional attributes and the crude fiber content was reported as; *eup* 6.69%; *hecche* 18.66%; *hirring* 25.88%; *soidon* 20.65%; *ekung* 24.62% and *soibum* 23.61% (Sonar et al., 2015). The crude fibre content in *soibum* as reported by Singh et al. (2011) was in the range of 0.35-0.60 g/100g. Choudhury et al. (2011) reported the presence of crude fibre in processed bamboo shoots to be 1.8 g/100g.

Crude fibre is naturally present in vegetables and the amount and composition of fibres differ from food to food (Rodríguez et al., 2006). Several non-starch foods provide

upto 20–35 g of fibre per 100 g dry weight and those containing starch about 10 g per 100 g of dry weight; and the content of fibre of fruits and vegetables is 1.5–2.5 g per 100 g of dry weight (Selvendran and Robertson, 1994). In the present study the crude fiber in *anishi* was found to be 12.26 g/100g, which when compared to the above data seems to contain high amount of fibre. Crude fibre in *hungrii* was also found to be 1.02 g/100g, which decreased from its raw material having 2.88 g/100g, as *Brassica* leaves are considered to have high amount of fibre content (Gupta and Wagle, 1988). The crude fibre content in cucumber was relatively low and it decreased further after fermentation in *tsutuocie*. The reduction of crude fibre content in diet might be due to enzymatic degradation of the fibrous material during fermentation (Tufa et al., 2016).

During fermentation, optimal *p*H conditions are needed to be maintained for the microorganisms to act for enzymatic degradation of the anti-nutritional factors or to hydrolyze complex polyphenols into much simpler and active polyphenols (Cheng and Han, 2013). In the present study the *p*H of *axone/akhuni* and *tsutuocie* increased after fermentation, from 6.8 to 8 in *axone/akhuni* and from 6.5 to 8.2 respectively. The increase in *p*H that led to decrease in acidity of the fermented products can be attributed to the proteolysis and the release of ammonia through deaminase activity (Odunfa and Oyeyiola, 1985). Similar result was also observed in *kinema*, with *p*H increasing from 6.7 to 7.9 (Sarkar et al., 1994; Tamang, 2010). General et al. (2011) also reported the increase of *p*H from 6.0 to 8.6 in *hawaijar*. Tamang et al. (2012) also reported *tungrymbia* and *bekang* to be alkaline in nature with a *p*H of 7.6 and 7.1 respectively. Dajanta et al. (2012) reported the alkaline *p*H of *Thua Nao* (7.08-8.25) to be a typical characteristic of the product resulting from the basis end components especially ammonia via proteolysis of fermented organisms in soybean.

The fermented vegetable based products *anishi*, *hungrii* and *rhujuk/bastanga* had *p*H of 5.8, 5.2 and 4.7 respectively, which may be due to acids produced by microorganisms during fermentation preventing the growth of contaminating microorganisms (Breidt et al., 2013). Similar results were observed in *goyang, gundruk, sinki* and *khalpi* with *p*H of 6.5, 5.0, 4.1 and 3.9 respectively (Tamang, 2010). Bamboo shoot based fermented products like *eup, soibum, hirring, soidon, hecche* and *ekung,* were also reported to have *p*H of 3.9, 4.3, 4.3, 5.3, 4.2 and 4 respectively (Sonar et al., 2015).

The variation in the levels of proximate composition of foods after fermentation may be influenced by various factors like the different varieties of raw material used or the influence of environmental factors and also on the conditions involved during its processing (Fernandes et al., 2016).

Total phenolic and flavonoid content

Bioactive compounds are found naturally in most plants and the majority of natural antioxidants are phenols and flavonoids. High intake of these compounds endowed with antioxidant and anti-inflammatory activity may have positive impact on human health, especially in the prevention of cancer and inflammatory diseases (Pisoschi and Negulescu, 2011). Depending on the pH and temperature conditions, a fermentation process may dramatically modify the content and the composition of these bioactive compounds (Mathias et al., 2006). In soybeans, phenolic compounds are one of the major groups of compounds acting as a primary antioxidant or free radical scavenger (Shahidi et al., 1992). In the present study the total phenolic and flavonoid content of *axone/akhuni*, were found to be 0.86mg GAE/g and 0.64 mg GAE/g respectively (Moktan et al., 2008) reported the total phenolic content in *kinema*, having 3.386mg GAE/g, which was much higher than the value reported in the present study. Chonkeeree et al. (2013) reported

increase in total phenolic content in the dried fermented soybean product than that of the non-fermented soybean extract as reported in the present study. Shon et al. (2007) reported the increase in the total polyphenol during the 3-day fermentation to 22.19mg/g of dry weight and 8.81mg/g of dry weight of *Chungkookjang* and steamed soybeans, respectively. The total pehonlic and flavonoid content in *anishi* (1.44mg GAE/g and 2.06mg QE/g) and *rhujuk/bastanag* (2.44mg GAE/g and 0.62mg QE/g), were significantly higher than in the raw materials. Sonar et al. (2015) reported the presence of total phenolic and flavonoid content in fermented bamboo shoots of North East, India and the highest phenolic content was observed in *eup* (920 µg/g), whereas, the lowest phenolic content (718.03 µg/g) was observed in *soidon*. The highest total flavonoid content was observed in *hirring* (308.72 µg/g) and lowest in *eup* (568.54 µg/g). Fermentation have been reported to increase the phenolic and flavonoid content by inducing structural breakdown of the substrate cell wall leading to release of bioactive in plant based functional foods (Ibrahim et al., 2014). However, the total phenolic and flavonoid content in *hungrii* (1.66 mg GAE/g and 0.76 mg QE/g), decreased from its raw material. The total polyphenol content of *kimchi*, prepared from mustard leaves showed 69.67–122.67 mg GAE/100 g extract and the total flavonoid of 76.0–82.33 mg QE/100 g extract content during 24 days of fermentation (Oh et al., 2017). The total flavonoid content of cabbage *kimchi* extract fermented for 5 and 24 days increased gradually from 4.1–6.5 mg QE/g extract on day 0 to 11.7–12.6 mg QE/g extract on day 24 (Jung et al., 2016). The total phenolic and flavonoid content in *tsutüocie* (0.22mg GAE/g and 0.1mg QE/g) had relatively lower levels of phenolics as compared to its counterpart, the reason for the decrease in the level of bioactive compounds may be due to strengthening of plant cell walls into lignans and lignins by polymerisation (Wu et al., 2011).

Antioxidant activity

Antioxidants are substances which inhibits or delay oxidative processes which occur due to influence of atmospheric oxygen or reactive oxygen species (Pisoschi and Negulescu, 2011). During fermentation, bacterial enzyme transforms organic substances into simpler compounds such as peptides, amino acids and other nitrogenous compounds which not only contribute to the flavour and aroma of the fermented products but some exhibit antioxidant capacity (Joshi and Biswas, 2015). Higher the content of phenolic substances with antioxidant ability, the greater the scavenging activity (Nicoli et al., 1999). The assay of the scavenging of DPPH radical is a rapid, simple and inexpensive method widely used to evaluate the antioxidant capacity of extracts from different sources. Unlike other free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Shekhar and Anju, 2014). The essence of DPPH assay is that the antioxidant react with the stable free radical 1,1-Diphenyl-2-picrylhydrazyl (deep violet colour) and converts it to 1,1-Diphenyl-2 picrylhydrazine with a yellow colour. The degree of discoloration indicates the scavenging potential of the sample antioxidant resulting in a decrease in absorbance at 517 nm (Pisoschi and Negulescu, 2011). Free radical scavenging activity for DPPH radical was expressed as IC50 value (the concentration required to scavenge 50% of DPPH). The free radical scavenging activity of *axone/akhuni*, was found to be 98.79 µg/ml, which increased from its raw material soybean seeds having 186.75 µg/ml. The total antioxidant effect of collected *axone/akhuni* samples in this study are weaker than the inhibition effects that were reported in *thuo nao* (Dajanta et al., 2013), *Chungkukjang* (Shon et al., 2007) and *Koji* (Lee et al., 2007).

The free radical scavenging activity of *anishi* (60.2 µg/ml) and *rhujuk/bastanga* (92.85 µg/ml) were higher than in colocasia leaves (100.9 µg/ml) and young succulent bamboo shoots (112.3 µg/ml). However, the free radical scavenging activity of *tsutuocie* (219.3 µg/ml) was lower compared to cucumber fruit (120 µg/ml). Sonar et al. (2015) reported the increase in antioxidant activity of fermented bamboo shoots with time in fermentation. Diverse fermentation processes of fermented bamboo shoots and their method of preparation could contribute for the higher antioxidant activity. Oh et al. (2017) reported that during *kimchi* manufacturing process the antioxidant components contained in leaf mustard were degraded, similarly in the present study *hungrii* (73.3 µg/ml) had lower antioxidant activity than brassica/mustard leaves (65.7 µg/ml).

The antioxidant activity of the fermented products showed a significant correlation between the concentration of phenolic compounds and the scavenging activity of DPPH. It may not only depend on the concentration of phenolic compounds but also on the kind of phenolic compounds which varies with the degree of hydroxylation and polymerisation (Yadav et al., 2016). Microorganisms during fermentation are exposed to oxidative stress making the cells evolve protective mechanisms involving enzymatic antioxidation, which may contribute to the antioxidative effect of fermentation (Hur et al., 2014). High antioxidant activity might be due to fermentation process and also biochemical changes that could promote binding of dietary fiber to polyphenols followed by decomposition into free phenolic compounds (Aikpokpodion and Dongo, 2010.). Diverse fermentation processes and their method of preparation could alter the availability of antioxidant activity in the different fermented product (Sonar et al., 2015).

Summary and Conclusion

Food composition data are necessary to be considered from a Nutritionist's viewpoint. It provides valuable information of nutritive value of the food products. In

addition, these data can be used as nutritional standard or as the basis recommendation for Government's health policy. Thus, this chapter deals with the nutritional analysis of five major fermented food products of Nagaland i.e., *axone/akhuni, anishi, hungrii, rhujuk/bastanga* and *tsutuocie,* in comparison to its raw materials. In the proximate analysis, *axone/akhuni, tsutuocie* and *rhujuk/bastanga* had higher levels of moisture as compared to its raw materials. The protein content in *axone/akhuni, anishi* and *hungrii,* increased from that of its raw material. The percentage of reducing sugars increased in *axone/akhuni, hungrii* and *tsutuocie* in comparison to the raw material. The crude fibre content in *axone/akhuni, anishi* and *rhujuk/bastanga* increased from that of the raw material. *Anishi, hungrii* and *rhujuk/bastanga* had low *p*H and were acidic in nature, while *axone/akhuni* and *tsutuocie* were found to be alkaline in nature with high *p*H. The total phenolic content in *axone/akhuni, anishi* and *rhujuk/bastanga,* increased from that of its raw material. The total flavonoid content in *axone/akhuni, anishi, rhujuk/bastanga* and *tsutuocie,* increased compared to its raw material. Antioxidant activity of *axone/akhuni, anishi* and *rhujuk/bastanga,* were found to be higher than its raw material. Result from this study demonstrates that most of the fermented food products were rich in nutrients in comparison to its raw material and thus, their proper utilization, exploitation and conservation is of utmost importance. However, in *tsutuocie* the levels of some major nutrients tend to decline, which may be due to the nature of the fermentation technique leading to natural leaching of some solubilised components (Paredes-Lopez and Harry, 1988). Fermented products *axone/akhuni, anishi, hungrii* and *rhujuk/bastanga* were found to be good source of protein; *axone/akhuni, anishi* and *hungrii* had good amount of crude fiber; *axone/akhuni, anishi* and *rhujuk/bastanga* were found to be good source of total phenolic and flavonoid content, thus having high levels of antioxidant activity. Therefore, it can be concluded that fermentation helps in the improvement of the

nutritional profile of these fermented products, which can contribute to the dietary status of consumers, leading to improvement in product acceptability (Oguntoyinbo et al., 2016). Increasing health awareness among the human population is creating a genuine need for adopting a nutritionally complete custom-made diet, taking into account convenience, cost, taste and availability in the food market (Chandramouli and Viswanath, 2015). Studied fermented products are nutraceutically important and offer a role to play in service of society as a potential source of nutritional and nutraceutical components. Hence, there is a need to improvise the quality of these fermented foods for commercialization and human consumption, which needs to be taken up in the near future.

Chapter - 5

Summary and Conclusion

Food is one of the basic needs of man. Consumption of food besides satisfying hunger and promoting growth and energy to the body, enhances friendliness and social warmth. Community food security is a strategy for ensuring secure access to adequate amounts of safe, nutritious, culturally appropriate food for everyone, produced in an environmentally sustainable way, and provided in a manner that promotes human dignity. Every society or group has its own conception of food and own history of food habits, rather indigenous, which shape their food culture. Food is strongly connected to the culture of a community and provides it with a distinct identity. Fermentation is one of the oldest methods of food preservation in the world. It is the transformation of the simple raw materials into value added products by microorganisms or their enzymes on various substrates. Traditional or indigenous fermented foods are those popular products that since early history have formed an integral part of the diet and that can be prepared in the household or in cottage industry using relatively simple techniques and equipment.

The major objective of this study was to document indigenous knowledge of ethnic people of Nagaland on production of some of the popularly consumed fermented

food products. Beside this, the other objective was to analyse the nutritional value and to isolate, characterise and identify the dominant microorganisms of five fermented food products *viz., axone/akhuni, anishi, hungrii, rhujuk/bastanga* and *tsutuocie*.

During the study, survey was conducted in different regions in Nagaland and various indigenously prepared fermented foods and beverages of Nagaland were documented based on personal observation and interviews with the local people (producers). Nagaland inhabited by diverse tribal communities produces varieties of fermented foods and beverages. The fermented food products produced by the ethnic people using their native knowledge of preservation of perishable raw materials without using starter culture and chemicals, was found both as low-cost ethnic foods and beneficial for socio-cultural upliftment of the people. Most of the fermented food and beverages in Nagaland are associated with a particular tribe and in a way give a cultural identification value for communities through its food. The different types of traditional fermented foods and beverages of this state are unique from the other states and the people of this state have preserved the taste for fermentation products and processes for the production of fermented foods from generations on. The fermented foods and beverages documented in this study are *zutho, axone/akhuni, anishi, Jang kap, hungrii, rhujuk/bastanga, jangpangngatsu, tsutuocie,* fermented pork fat and fermented fruit beverages. These fermented food products form an important component of the staple diet of the people in Nagaland.

A total of 25 samples of *axone/akhuni, anishi, hungrii, rhujuk/bastanga* and *tsutuocie* were analyzed for their microbial population. In *axone/akhuni* and *rhujuk/bastanga,* the total viable microbial load was in the range of 10^7 cfu ml^{-1}. However, in *anishi, hungrii* and *tsutuocie,* the total viable microbial load was low in the range of 10^4 cfu ml^{-1}, which may be due to the pre or post fermentation treatment of

drying and addition of water creating an environment suitable for the growth of only particular microorganisms. No yeast or moulds were detected in any of the samples. On the basis of a combination of phenotypic and genotypic characterization, *Bacillus* species was found to be the most dominant microorganism present in almost all the fermented food products studied for its microbial population.

The different groups of microorganisms were identified by sequencing the partial 16S rRNA and comparing their sequence data to sequences listed in the NCBI database. In *axone/akhuni*, the identified *Bacillus* species were *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus cereus*, followed by *Staphylococcus* species and *Alcaligenes faecalis*. Pathogenic bacteria like *Bacillus cereus, Staphylococcus* sp. and *Alcaligenes faecalis* were detected in few samples, which might have been introduced during handling of raw materials during the preparation process. In *anishi*, the identified *Bacillus* species were *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilis*, followed by *Enterococcus faecalis*. In *hungrii*, the identified *Bacillus* species were *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilis* and *Bacillus amyloliquefaceins.* In *rhujuk/bastanga*, the identified *Bacillus* species were *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaceins*, followed by *Staphylococcus* species. In *tsutuocie*, the identified *Bacillus* species were *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilis.*

Many fermented foods are now receiving world attention for their health-promoting or disease-preventing effects. The nutritional composition of ethnic fermented foods showed the nutritional value essential for local people in their diet. The same group of fermented products *i.e.*, *axone/akhuni*, *anishi*, *hungrii*, *rhujuk/bastanga* and *tsutuocie* have been evaluated for nutritional components which included estimation of moisture, protein, reducing sugars, crude fibre, total phenol content, total flavonoid content and

antioxidant activity. *Axone/akhuni* and *tsutuocie* had higher moisture than its substrate. The respective contents of protein, reducing sugars and crude fibre in *axone/akhuni* were higher than those of the raw material. In *tsutuocie*, protein, reducing sugars and crude fibre content decreased from that of its raw material. *Axone/akhuni* and *tsutuocie* were found to be alkaline in nature with a *p*H of 8.0 and 8.2. *Anishi* and *hungrii* had lower moisture than its substrates. The fermentation of *Colocasia* and *Brassica* leaves caused a decrease in *p*H (5.8 and 5.2). In the fermented product *anishi,* protein and crude fibre content increased but the reducing sugars decreased than that of its substrate. In *hungrii*, the protein content and reducing sugars increased but the crude fibre decreased than its substrate. *Rhujuk/bastanga* was acidic (4.7) and had higher moisture content than its raw material. While the content of crude fibre of *rhujuk/bastanga* was significantly higher than those of its substrate, the protein content and reducing sugars of the substrate was higher than those of the product.

Total phenol content and total flavonoid content in the fermented product *axone/anishi*, *anishi* and *rhujuk/bastanga* increased in comparison to its raw material, thus increasing the DPPH-scavenging activity of the fermented products than its substrates. In *hungrii* and *tsutuocie*, the DPPH-scavenging activity decreased as compared to its raw material due to low content of total phenol and total flavonoid in the fermented products. Proximate composition and antioxidants are found to be in substantial amount in most of the fermented food products evaluated presently. Hence studied species are nutraceutically important and offers a role to play in service of society as a potential source of nutritional components.

Conclusion

In the present study documentation of some popular fermented food was done along with nutritional analysis. The dominant microorganisms present in these fermented

foods were identified based on morphological and molecular markers. The findings of the present study will help in popularizing these ethnic foods at larger platform/market. In the present study few microbes were isolated which are considered to be undesired in foods. Presence of contaminants in the fermented products may be due to poor techniques of manufacture or contamination of the product from wash water, equipment and other sources during the processing products. Hence, hygienic practices must be taught to the persons involved in handling and processing of these products. Further, more detailed investigation on the microorganisms and their role in the nutrition and health value of the food is needed. Also, starter cultures with desired microorganisms are required to accelerate the fermentation process as well as to improve the quality of the fermented product.

References

Abegaz, K. 2007. Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of borde, an Ethiopian cereal beverage. *Afr J Biotech*. 6(12): 1469-1478.

Achi, O. K. 2005. The Upgrading of traditional fermented foods through biotechnology. *Afr J Biotech*. 4: 375-380.

Achi, O. K., Anokwuru, I. C. and Ogbo, F. C. 2007. Microbiological and chemical changes during fermentation of crabs for ogiri-nsiko production. *Ame J Food Technol*. 2(4): 301-306.

Adams, M. R. and Nout, M. J. R. 2001. Fermentation and Food safety. Aspen Publishers, Gaithersburg, Md.

Adam, M. 2010. Fermented meat products. In: (Tamang, J. P. and Kailasapathy, K. eds.) *Fermented Foods and Beverages of the World*. CRC Press, pp: 309-322.

Adeyemo, S. M. and Onilude, A. A. 2014. Molecular identification of *Lactobacillus plantarum* isolated from fermenting cereals. *Intl J Biotech Mol Biol Res*. 5(6): 59-67. DOI: 10.5897/IJBMBR2014.0184.

Agrahar-Murugkar, D. and Subbulakshmi, G. 2006. Preparation technique and nutritive value of fermented foods from the Khasi tribes of Meghalaya. *Ecol Food Nutr*. 45: 27-38.

Aidoo, K. E., Nout, N. J. R. and Sarkar, P. K. 2006. Occurrence and function of yeasts in Asian indigenous fermented foods. *FEMS Yeast Res.* 6(1): 30-39.

Aikpokpodion, P. E. and Dongo, L. N. 2010. Effects of fermentation intensity on polyphenols and antioxidant capacity of cocoa beans. *Intl J Sustainable Crop Prod.* 5(4): 72-76.

Aka, S., Konan, G., Fokou, G., Dje, K. M. and Bonfoh, B. 2014. Review on African traditional cereal beverages. *Ame J Res Comm. 2*(5): 103-153.

Al-Jalay, B., Blank, G., McConnell, B. and Al-Khayat, M. 1987. Antioxidant activity of selected spices used in fermented meat sausage. *J Food Protection.* 50(1): 25-27.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. 1990. Basic local alignment search tool. *J Mol Biol*. 215(3): 403-410.

Amoa-Awua, W. K., Terlabie, N. N. and Sakyi-Dawson, E. 2006. Screening of 42 *Bacillus* isolates for ability to ferment soybeans into *dawadawa. Intl J Food Microbiol.* 106: 343-347.

Amadou, I., Yong-Hui, S., Sun, J. and Gou-Wei, L. 2009. Fermented soybean products: some methods, antioxidant compound extraction and their scavenging activity. *Asian J Biochem.* 4(3): 68-76.

Aneja, K. R. 2003. *Experiments in Microbiology*, *Plant Pathology and Biotechnology*. New Age International, Ansari Road, Daryaganj, New Delhi.

Arai, S. 1996. Studies on functional foods in Japan-state of the art. *Biosci Biotech Biochem*. 60(1): 9-15. DOI: 10.1271/bbb.60.9.

Ari, M. M., Ayanwale, B. A., Adama, T. Z. and Olantunji, E. A. 2012. Effects of different fermentation methods on the proximate composition, amino acid profile and some antinutritionla factors (ANFs) in soyabeans (*Glycine max*). *J Fermen Technol Bioeng.* 2: 6-13.

Arjun, J., Verma, A. K. and Prasad, S. B. 2014. Method of preparation and biochemical analysis of local tribal wine *Judima:* an indegenous alcohol used by Dimasa tribe of North Cachhar Hills District of Assam, India. *Intl Food Res J.* 21(2): 463-470.

Azokpota, P., Hounhouigan, D. J. and Nago, M. C. 2006. Microbiological and chemical changes during the fermentation of African locust bean (*Parkia biglobosa*) to produce

afitin, iru, and sonru, three traditional condiments produced in Benin. *Intl J Food Microbiol.* 107: 304–309.

Beaumont, M. 2002. Flavouring composition prepared by fermentation with *Bacillus* spp. *Intl J Food Microbiol*. 75: 189-196.

Baek, J. G., Shim, S. M., Kwon, D. Y., Choi, H. K., Lee, C. H. and Kim, Y. S. 2010. Metabolite profiling of Cheonggukjang, a fermented soybean paste, inoculated with various *Bacillus* strains during fermentation. *Biosci Biotech Biochem*. 74(9): 1860-1868.

Bahareh H. Sarmadi, B. H. and Amin Ismail, A. 2010. Antioxidative peptides from food proteins: A review. *Peptides.* 31: 1949–1956

Bajwa, H. R., Nirmala, C., Koul, A. and Bisht, M. S. 2016. Changes in organoleptic, physicochemical and nutritional qualities of shoots of an edible bamboo *Dendrocalamus hamiltonii* Nees and Arn. Ex Munro during processing. *J Food Process Preserv.* 40(6): 1309-1317.

Bates, L. D., Iwarden, P. V., Meeus, N., Schimmel, H., Philipp, W. and Emons, H. 2008. First certified reference material for molecular finger printing of two approved probiotic *Bacillus* strains, European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Retieseweg, Belgium. pp: 111-2440.

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

Battcock, M. and Azam-Ali, S. 1998. *Fermented Fruits and Vegetables. A Global Perspective*. FAO Agricultural Services Bulletin No.134, Rome, Italy.

Basumatary, A., Middha, S. K., Usha, T., Basumatary, A. K., Brahma, B. K. and Goyal, A. K. 2017. Bamboo shoots as a nutritive boon for Northeast India: an overview. *Biotech*. 7(3): 169.

Beddows, C. G. 1985. Fermented fish and fish products. In: (Wood, B. J. B., ed.) *Microbiology of Fermented Foods*, Vol. 2. Elsevier Applied Sci. Publ., London, pp. 1–40.

Bhatt, B. P., Singha, L. B., Singh, K., and Sachan, M. S. 2003. Some commercial edible bamboo species of Norteast India: Production, indigenous uses, cost-benefit and management strategies. *Bamboo Sci Cult.* 17(1): 4–20.

Bisen, P. S., Mousumi, D. and Prasad, G. B. K. S. 2012. Microbes as a tool for industry and research. In: *Microbes-Concepts and Applications*. Wiley Blackwell, London, pp. 681–567.

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

Bottone, E. J. and Peluso, R. W. 2003. Production by *Bacillus pumilus* (MSH) of an antifungal compound that is active against Mucoraceae and *Aspergillus* species: preliminary report. *J Med Microbiol*. 52: 69-74. DOI: 10.1099/jmm.0.04935-0.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powel, I. B., Prajapati, J. B., Seto, Y., Schure, E. T., Boven, A. V., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S. and Hansen, E. B. 2012. Food fermentations: microorganisms with technological beneficial use. *Intl J Food Technol*. 154(3): 87-97.

Breidt, F., Pérez-Díaz, I., McFeeters, R. F. and Lee, C. H. 2013. Fermented vegetables. In: (Doyle, M. P. and Buchanan R. L. eds.) *Food Microbiology: Fundamentals and Frontiers, 4th edition*. ASM Press, Washington, D.C. pp: 841-855.

Breidt, F., Pérez-Díaz, I., McFeeters, R. F. and Lee, C. H. 2013. Fermented vegetables. In: (Doyle, M. P. and Buchanan R. L., eds.) *Food Microbiology: Fundamentals and Frontiers* (4th ed.), ASM Press, Washington, D.C. pp. 841-855. DOI: 10.1128/9781555818463.ch33.

Brosnan, J. 2003. Interorgan amino acid transport and its regulation. *J Nutr*. 133: 2068-2072.

Buckenhüskes, H. J. 1993. Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. *FEMS Microbiol Rev.* 12(1-3): 253-271. DOI: org/10.1016/0168-6445(93)90067-J.

Buragohain, J., Angami, T., Choudhary, B. U., Singh, P. and Bhatt, B. P. 2013. Quality evaluation of indigenous Taro (*Colocasia esculenta* L.) cultivars of Nagaland. *Indian J Hill Farming.* 26(2): 16-20.

Callewaert, R., Hugas, M. and DeVuyst, L. 2000. Competitiveness and bacteriocin production of *Enterococci* in the production of Spanish-style dry fermented sausages. *Intl J Food Microbiol*. 57(1-2): 33-42.

Campbell-Platt, G. 1987. *Fermented Foods of the World-A Dictionary and Guide.* Butterworths, London.

Campbell-Platt, G. 1994. Fermented foods-a world prespective. *Food Res Intl.* 27: 253-257.

Campbell-Platt, G. 1995. Fermented meats-a world perspective. In: (Campbell-Platt, G. and Cook, P. E., eds) *Fermented Meats*. Springer, USA, pp. 39-52.

Campbell-Platt, G. and Cook, P. E. 1995. *Fermented Meats*, Blackie Academic & Professional, Glasgow.

Caplice, E. and Fitzgerald, G. F. 1999. Food fermentations: role of microorganisms in food production and preservation. *Intl J Food Microbiol*. 50: 131-149.

Chakrabarty, J. and Kharel, N. 2012. Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East, India. *Indian J Trad Knowl.* 11(1): 7-25.

Chakrabarty, J., Sharma, G. D. and Tamang, J. P. 2009. Substrate utilisation in traditional fermentation technology practiced by Tribes of North Cachar Hills district of Assam. *J Sci Technol Biol Sci.* 4(1): 66-72.

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

Chandramouli, S. and Viswanath, S. 2015. Nutritional composition of edible bamboo shoots of some commercially important bamboo species in Peninsular India. *Intl J Basic Life Sci.* 3(6): 275- 287.

Chavan, J. K., Kadam, S. S. and Beuchat, L. R. 1989. Nutritional improvement of cereals by fermentation. *Critical Rev Food Sci Nutr*. 28(5): 349-400.

Cheigh, H. and Park, K. 1994. Biochemical, microbiological, and nutritional aspects of kimchi (Korean fermented vegetable products). *Crit Rev Food Sci Nutrition.* 34 (2): 175–203.

Chelule, P. K., Mbongwa, H. P., Carries, S. and Gqaleni, N. 2010. Lactic acid fermentation improves the quality of amahewu, a traditional South African maize-based porridge. *Food Chem.* 122(3): 656-661.

Chen, K., Erh, M., Su, N., Liu, W., Chou, C. and Cheng, K. 2012. Soyfoods and soybean products: from traditional use to modern applications. *Appl Microbiol Biotech*. 96: 9-22.

Chen, Y. S., Wu, H. C., Lo, H. Y., Lin, W. C., Hsu, W. H., Lin, C. W., Lin, P. Y. and Yanagida, F. 2012. Isolation and characterisation of lactic acid bacteria from jiang□gua (fermented cucumbers), a traditional fermented food in Taiwan. *J Sci Food Agricult.* 92(10): 2069-2075.

Chen, B., Wu, Q. and Xu, Y. 2014. Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese Maotai-flavor liquor. *Intl J Food Microbiol*. 179: 80–84.

Chettri, R. and Tamang, J. P. 2015. *Bacillus* species isolated from *tungrymbai* and *bekang*, naturally fermented soybean foods of India. *Intl J Food Microbiol*. 197: 72-76.

Chhay, T. Y., Borin, K., Preston, T. R. and Sokveasna, M. 2007. Intake, digestibility and N retention by growing pigs fed ensiled or dried Taro (*Colocasia esculenta*) leaves as the protein supplement in basal diets of rice bran/broken rice or rice bran/cassava root meal. *Livestock Res Rural Dev*. 19: (9). DOI:lrrd.org/lrrd19/9/chha19137.htm.

Chonkeeree, A. and Chumchuere, M. C. S. 2013. Antioxidant Activity and Total Phenolic Content of Dried Fermented-Soybean Products Fermented with *Bacillus subtilis* and Lab: Potential for functional food application. *Intl Proc Chem Biol Env Eng.* 58(4): 16-21.

Choudhury, D., Sahu, J. K. and Sharma, G. D. 2011. Bamboo shoot based fermented food products: a review. *J Sci Ind Res.* 70: 199-203.

Choudhury, D., Sahu, J. K. and Sharma, G. D. 2012. Bamboo shoot: microbiology, biochemistry and technology of fermentation. *Indian J Trad Knowl.* 11(2): 242-249.

Choudhury, D., Sahu, J. K., and Sharma, G. D. 2012. Value addition to bamboo shoots: A review. *J Food Sci Technol*. 49(4): 407–414.

Christine Hotz, C. and Gibson, R. S. 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *J Nutrition.* 137: 1097-1100.

Chukeatirote, E. 2015. Thua nao: Thai fermented soybean. *J Ethnic Foods*. 2: 115-118.

Cocolin, L., Bisson, L. F. and Mills, D. A. 2000. Direct pro¢ling of the yeast dynamics in wine fermentations. *FEMS Microbiol Let*. 189: 81-87.

Cocolin, L., Alessandria, V., Dolci, P., Gorra, R. and Rantsiou, K. 2013. Culture independent methods to assess the diversity and dynamics of microbiota during food fermentation. *Intl J Food Microbiol*. 167: 29-43.

Cogan, T.M. and Hill, C. 1993. Cheese starter cultures. In: (Fox, P.F., ed.) *Cheese: Chem Phys Microbiol*. Vol. 1, 2nd Ed. Chapman and Hall, London, pp. 193–255.

Cogan, T. M. and Accolas, J. P. 1996. *Dairy Starter Cultures*. VCH Publishers, Cambridge.

Conner, H. A. and Allgeier, R. J. 1976. Vinegar: Its history and development. *Adv Appl Microbiol*. 20: 81–133.

Czerucka, D., Piche, T. and Rampal, P. 2007. Yeast as probiotics –*Saccharomyces boulardii*. *Aliment Pharm Ther*. 26(6): 767-778.

Daha, N. R., Karki, T. B., Swamylingappa, B., Li, Q. and Gu, G. 2005. Traditional foods and beverages of Nepal-A review. *Food Reviews Intl*. 21(1): 1-25. DOI: 10.1081/FRI-200040579.

Dajanta, K., Chukeatirote, E., Apichartsrangkoon. A. and Frazier, R. A. 2009. Enhanced aglycone production of fermented soybean products by *Bacillus* species. *Acta Biologica Szegediensis*. 53(2): 93-98.

Dajanta, K., Janpum, P. and Leksing, W. 2013. Antioxidant capacities, total phenolics and flavonoids in black and yellow soybeans fermented by *Bacillus subtilis*: A

comparative study of Thai fermented soybeans (thua nao). *Intl Food Res J.* 20(6): 3125-3132.

Dahal, N., Karki, T. B., Swamylingappa, B., Li, Q. and Gu, G. 2005. Traditional foods and beverages of Nepal-A review. *Food Rev Intl.* 21: 1-25.

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

Di Cagno, R., Coda, R., De Angelis, M. and Gobbetti, M. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol*. 33(1): 1-10.

Dixit, U. and Goyal, V. C. 2011. Traditional Knowledge from and for elderly. *Indian J Trad Knowl*. 10(3): 429-438.

Dubnau, D., Smith, I. and Marmur, J. 1965. Gene conservation in *Bacillus* species, I. The location of genes concerned with the synthesis of ribosomal components and soluble RNA. *Proc Nat Acad Sci USA*. 54: 491-498.

Dutta, B. K. and Dutta, P. K. 2004. Potential of ethnobotanical studies in North East India: An overview. *Indian J Trad Knowl*. 4(1): 7-14.

Ekumankama, I. O. 2008. Nutrient composition of indigenous vegetables (*Pterocarpus soyanxii, Pterocarpus santalinoides* and *Gnetum africanum*). *Nige J Nutr Sci*. 29: 195-200.

Elango, R. and Laviano, A. 2017. Protein and amino acids: key players in modulating health and disease. *Curr Opinion Clin Nutri Metabolic Care.* 20(1): 69–70. DOI: 10.1097/MCO.0000000000000343.

Enujiugha, V. N. and Badejo, A. A. 2017. Probiotic potentials of cereal-based beverages. *Critical Rev Food Sci Nutr*. 57(4): 790-804.

Erbas, M., Ertugay, M. F., Erbas, M. O. and Certel, M. 2005. The effect of fermentation and storage on free amino acids of *tarhana*. *Intl J Food Sci Nutr*. 56 (5): 349–358.

Essiett, U. A. and Ukpong, U. J. 2014. Comparative phytochemical, nutrient and anti-nutrient of stems of *Ipomoea involucrata* Beauv, *Ipomoea. Triloba* L. and *Ipomoea batatas* Lam. *Ame J Food Nutr.* 2(4): 71-76.

FAO. 1975. The state of Food and agriculture 1974. FAO Library AN: 129636. Rome, Italy.

FAO. 1996. The state of Food and agriculture. FAO Agriculture series, no. 39. ISBN 92-5-103858-9. Rome, Italy.

Farmworth, E. R. 2003. Handbook of Fermented Functional Foods. CRC Press, Boca Raton, Fla.

Fabiyi, E. B. 2006. Soyabean processing, utilization and health benefits. *Pakistan J Nutr.* 5(5): 453- 457.

Fernandes, R. P. P., Trindade, M. A., Tonin, F. G., Lima, C. G., Pugine, S. M. P., Munekata, P. E. S., Lorenzo, J. M. and Melo, M. P. 2016. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers**.** *J Food Sci Technol***.** 53: 451-460.

Flint, J. A., Duynhoven, Y. T. V., Angulo, F. J., DeLong, S. M., Braun, P., Kirk, M., Scallan, E., Fitzgerald, M., Adak, G. K., Sockett, P., Ellis, A., Hall, G., Gargouri, N., Walke, H. and Braam, P. 2005. Estimating the burden of acute gastroenteritis, food borne disease, and pathogens commonly transmitted by food: an international review. *Clinic Infectious Diseases*. 41: 698-704.

Font de Valdez, G., Gerez, C. L., Torino, M. I. and Rollan, G. 2010. New trends in cereal-based products using Lactic Acid Bacteria. In: (Mozzi, F., Raya, R. R. and Vignolo, G. M., eds.) *Biotechnology of Lactic Acid Bacteria: Novel Application*. Blackwell Publishing, New Jersey, United States, pp: 273-287.

Fujita, M., Nomura, K., Hong, K., Ito, Y., Asada, A. and Nishimuro, S. 1993. Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese natto, a popular soybean fermented food in Japan. *Biochem Biophysic Res Comm*. 197(3): 1340-1347.

Gadaga, T. H., Mutukumira, A. N., Narvhus, J. A. and Feresu, S. B. 1999. A review of traditional fermented foods and beverages of Zimbabwe. *Intl J Food Microbiol*. 53(1): 1-11.

Gates, K. and Costilow, R. N. 1981. Factors influencing softening of salt-stock pickles in air-purged fermentations. *J Food Sci*. 46(1): 274-282. DOI: 10.1111/j.1365-2621.1981.tb14580.x.

General, T., Koijam, K. and Appaiah K. A. A. 2011. Process improvement as influenced by inoculum and product preservation in the production of Hawaijar - A traditional fermented Soybean. *Afr J Food Sci.* 5(2): 63 - 68

Gibbs, B. F., Zougman, A., Masse, R. and Mulligan, C. 2004. Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. *Food Res Intl.* 37(2): 123-131.

Gibson, R. S., Perlas, L. and Hotz, C. 2006. Improving the bioavailability of nutrients in plant foods at the household level. *Proc Nutr Soc*. 65: 160–168. DOI: 10.1079/PNS2006489.

Giraffa, G. and Neviani, E. 2001. DNA-based, culture-independent strategies for evaluating microbial communities in food-associated ecosystems. *Intl J Food Microbiol*. 67: 19-34.

Giraffa, G. 2003. Bacteriocin and flavour production by *Enterococci*. *Intl J Food Microbiol*. 88: 215-222.

Giraffa, G. 2004. Studying the dynamics of microbial populations during food fermentation. *FEMS Microbiol Reviews*. 28: 251-260.

Giraffa, G., Chanishvili, N. and Widyastuti, Y. 2010. Importance of lactobacilli in food and feed biotechnology. *Res Microbiol.* 161: 480-487.

Gobbetti, M., Dicagno, R. and Angelis, M. D. 2010. Functional microorganisms for functional food quality. *Critical Rev Food Sci Nutr*. 50:716-727. DOI: 10.1080/10408398.2010.499770.

Gonfa, A., Foster, H. A. and Holzapfel, W. H. 2001. Field survey and literatures review on traditional fermented milk products of Ethiopia. *Intl J Food Microbio*. 68: 173-186.

Gomes, B. C., Esteves, C. T., Palazzo, I. C. V., Darini, A. L. C., Felis, G. E., Sechi, L. A., Franco, B. D. G. M. and De Martinis, E. C. P. 2008. Prevalence and characterization of *Enterococcus* spp. isolated from Brazilian foods. *Food Microbiol*. 25(5): 668-675. DOI: 10.1016/j.fm.2008.03.008.

Gorman, S. 2011. Lacto fermented vegetables and their potential in the developing world. *Food Chain*. 1(1): 87-94. DOI: 10.3362/2046-1887.2011.007.

Greppi, A., Rantsiou, K., Padonou, W., Hounhouigan, J., Jespersen, L., Jakobsen, M. and Cocolin, L. 2013. Determination of yeast diversity in ogi, mawè, gowé and tchoukoutou by using culture-dependent and -independent methods. *Intl J Food Microbiol*. 165: 84-88.

Gubag, R., Omoloso, D. A. and Owens, J. D. 1996. Sapal: a traditional fermented taro [*Colocasia esculenta* (L.) Schott] corm and coconut cream mixture from Papua New Guinea. *Intl J Food Microbiol*. 28(3): 361-367.

Gupta, K. and Wagle, D. S. 1988. Nutritional and antinutritional factors of green leafy vegetables. *J Agricult Food Chem*. 36(3): 472-474.

Halm, M., Osei-Yaw, A., Hayford, A., Kpodo, K. A. and Amoa-Awua, W. K. A. 1996. Experiences with the use of a starter culture in the fermentation of maize for 'kenkey' production in Ghana. *World J Microbiol Biotech*. 12(5): 531-536.

Handoyo, T. and Morita, N. 2006. Structural and functional properties of fermented soybean (Tempeh) by using *Rhizopus oligosporus*. *Intl J Food Properties*. 9(2): 347-355. DOI: 10.1080/10942910500224746.

Hansen, E. B. 2002. Commercial bacterial starter cultures for fermented foods of the future. *Intl J Food Microbiol.*78:119–131.

Harrigan, W. F. and McCane, M. E., 1976. *Laboratory Methods in Food and Dairy Microbiology. (2nd edn)*. Academic Press, London.

Hasan, M.N., Sultan, M.Z. and Mar-E-Um, M. 2014. Significance of fermented food in nutrition and food science. *J Scientific Res*, 6(2): 373-386.

Hesseltine, C. W. and Wang, H. L. 1980. The importance of traditional fermented foods. *BioSci*. 30:402–404.

Hasseltine, C.W. 1983. Microbiology of oriental fermented foods. *Annual Review Microbiol*. 37: 575-601.

Holzapfel, W. H. 1997. Use of starter cultures in fermentation on a household scale. *Food Control*. 8: 241-258.

Holzapfel, W. H. and Franz, C. M. A. P. 2016. Produce from Africa's gardens: potential for leafy vegetables and fruit fermentations. *Frontiers Microbiol.* 7: 1-14.

Holzapfel, W. H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Intl J Food Microbiol*. 75: 197-212.

Hosoi, T. and Kiuchi, K. 2003. Natto–A food made by fermenting cooked soybeans with Bacillus subtilis (natto). In: (Farnworth, E.R., ed) *Handbook of Fermented Functional Foods.* pp.227-245.

Hounhouigan, D. J., Nout, M. J. R., Nago, C. M., Houben, J. H. and Rombouts, F. M. 1994. Microbiological changes in mawe during natural fermentation. *World J Microbiol Biotech.* 10(4): 410-413.

Hubert, J., Berger, M., Nepveu, F., Paul, F. and Dayde´, J. 2008. Effects of fermentation on the phytochemical composition and antioxidant properties of soy germ. *Food Chem.* 109: 709–721.

Huth, P. J. and Park, K. M. 2012. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. *Adv Nutr.* 3(3): 266-285.

Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I. and Kim, G. B. 2014. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chem.* 160: 346–356.

Ibrahim, N. A., Mustafa, S. and Ismail, A. 2014. Effect of lactic fermentation on the antioxidant capacity of Malaysian herbal teas. *Intl Food Res J.* 21: 1483-1488.

Ijong, F. G. and Ohta, Y. 1996. Physicochemical and microbiological changes associated with bakasang processing - a traditional Indonesian fermented fish sauce. *Sci. Food Agricult.* 71(1): 69-74.

Ishige, N. 1993. Cultural Aspects of Fermented Fish Products in Asia. In: (Lee, C. H., Steinkraus, K. H. and Reilly, P. J. A. eds.) *Fish Fermentation Technology*. United Nations University Press, pp: 13-32.

Ismail, A. A., El-Nockrashy, S. A. and Khorshid, M. A. 1983. A beverage from separated buffalo milk fermented with kefir grains. *J Soc Dairy Technol.* 36: 117–118.

Iwuoha, C. I. and Eke, O. S. 1996. Nigerian indigenous fermented foods: their traditional process operation, inherent problems, improvements and current status. *Food Res Intl*. 29: 527-540.

Iyengar, S. H. 1950. *Lokopakara of Chavundarava*. Oriental Manuscripts Library, Madras, pp: 120–134.

Janssens, L., De Pooter., H. L., Schamp., N. M. and Vandamme, E. J. 1992. Production of flavours by microorganisms. *Process Biochem*. 27(4): 195-215.

Jamir, B. and Deb, C. R. 2014. Some fermented foods and beverages of Nagaland, India. *Intl J Food Ferm Tech*. 4(2): 87-92. DOI: 10.5958/2321-712X.2014.01314.3.

Jeyaram, K., Singh, W. M., Premarani, T., Devi, A. R., Chanu, K. S., Talukdar, N. C. and Singh, M. R. 2008. Molecular identification of dominant microflora associated with Hawaijar- A traditional fermented soybean (*Glycine max* (L.)) food of Manipur, India. *Intl J Food Microbiol*. 122: 259-268.

Jeyaram, K., Singh, A., Romi, W., Devi, A. R., Singh, W. M., Dayanidhi, H., Singh, N. R. and Tamang, J. P. 2009. Traditional fermented foods of Manipur. *Indian J Tradi Knowl*. 8(1): 155-121.

Jeyaram, K., Romi, W., Singh, A., Devi, A. R. and Devi, S. S. 2010. Bacterial species associated with traditional starter cultures used for fermented bamboo shoot production in Manipur state of India. *Intl J Food Microbiol*. 143: 1-8.

Jill, E., Clarridge III, J. E. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiol Reviews*. 17(4): 840-862. DOI: 10.1128/CMR.17.4.840–862.200.

Josephsen, J. and Jespersen, L. 2004. Handbook of food and beverage fermentation technology. In: (Hui, Y.H., Meunier-Goddik, L., Hansen, Å.S., Josephsen, J., Nip, W.K., Stanfield, P.S., and Toldrá, F. Eds) *Starter Cultures and Fermented Products.* Marcel Dekker, Inc., 270 Madison Avenue, New York, pp. 23–49.

Joshi, V.K., Bhutani, V.P. and Thakur, N. K. 1999. Composition and nutrition of fermented products. In: (Joshi, V.K. and Ashok, P. eds.) *Biotechnology: Food Fermentation*. Vol. I. Educational Publishers and Distributors, New Delhi, pp: 259–320.

Joshi, V.K. and Pandey, A. 1999. Biotechnology: Food fermentation. In: (Joshi, V.K. and Pandey, A. eds.) *Biotechnology: Food Fermentation*. Vol. I. Educational Publishers and Distributors, New Delhi. pp. 1–24.

Joshi, S.R. and Biswas, K. 2015. Antioxidants of Fermented Foods. In: (Tamang, J.P., etd) *Health Benefits of Fermented Foods and Beverages*. CRC press, London, New York, pp 553-561. DIO: 10.1201/b18279-20.

Juan, M. Y. and Chou, C. C. 2010. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis BCRC 14715. *Food Microbiol*. 27: 586-591.

Jung, S. J., Kim, M.J. and Chae, S. W. 2016. Quality and functional characteristics of kimchi made with organically cultivated young Chinese cabbage (olgari-baechu). *J Ethnic Foods*. 3: 150-158.

Kaur, I. P., Chopra, K. and Saini, A. 2002. Probiotics: potential pharmaceutical applications. *European J Pharm. Sci*. 15: 1–9.

Keishing, S. and Banu, A.T. 2013. Hawaijar- A fermented soya of Manipur, India. *IOSR-JESTFT*. 4(2): 29-33.

Kesik, M., Blagodatsky, S., Papen, H. and Butterbach, B. K. 2006. Effect of pH, temperature and substrate on N2O, NO and CO2 production by *Alcaligenes faecalis. J Appl Microbiol*. 101(3): 655-667.

Khan, I. and Kang, S. C. 2016. Probiotic potential of nutritionally improved Lactobacillus plantarum DGK-17 isolated from Kimchi–A traditional Korean fermented food. *Food Control*. 60: 88-94.

Kim, S. J. and Park, K. H. 1995. Antimicrobial activities of the extracts of vegetable kimchi stuff. *Korean J Food Sci Technol*. 27(2), 216-220.

Kim, Y., J. Y. Cho, J. H. Kuk, J. H. Moon, J. I. Cho, Y. C. Kim, and K. H. Park. 2004. Identification and antimicrobial activity of phenylacetic acid produced by Bacillus *licheniformis* isolated from fermented soybean, C*hungkook-jang*. *Curr Microbiol*. 48: 312-317.

Kim, M. and Chun, J. 2005. Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S r RNA gene analysis. *Intl J Food Microbiol.* 103: 91-96.

Kim, N. Y., Song, E. J., Kwon, D. Y., Kim, H. P. and Heo, M. Y. 2008. Antioxidant and antigenotoxic activities of Korean fermented soybean. *Food Chem Toxicol*. 46: 1184–1189.

Koroleva, N. S. 1988. Technology of kefir and komys. *IDF Bull*. 227: 96–100.

Krasowska, A., Biegalska, A., Augustyniak, D., Los, M., Richert, M. and Lukaszewicz, M. 2015. Isolation and characterization of phages infecting *Bacillus subtilis*. *BioMed Res Intl.* Article ID 179597. 1-10. DOI: org/10.1155/2015/179597.

Kullisaar, T., Yilmer, M., Mikelsaar, M., Vihalemm, T., Annuk, H., Kairane, C. and Kilk, A. 2002. Two antioxidative lactobacilli strains as promising probiotics. *Intl J Food Microbiol.* 72: 215–224.

Kumar, P. P., Begum, V. H. and Kumaravel, S. 2012. Mineral nutrients of 'pazhaya sadham': A traditional fermented food of Tamil Nadu, India. *Int J Nutr Metab.* 4(11): 151-152. DOI: 10.5897/IJNAM11.068.

Kumar, R. S., Kanmani, P., Yuvaraj, N., Paari, K. A., Pattukumar, V. and Arul, V. 2013. Traditional Indian fermented foods: a rich source of lactic acid bacteria. *Intl J Food Sci Nutr*. 64(4): 415-428.

Kwaka, C. S., Leeb, M. S. and Park, S. C. 2007. Higher antioxidant properties of *Chungkookjang*, a fermented soybean paste, may be due to increased aglycone and malonylglycoside isoflavone during fermentation. *Nutr Res*. 27: 719–727.

Kwon, G. H., Lee, H. A., Park, J. Y., Kim, J. S., Lim, J., Park, C. S., Kwon, D. Y. and Kim, J. H. 2009. Development of a RAPD-PCR method for identification of *Bacillus* species isolated from Cheonggukjang. *Intl J Food Microbiol.* 129: 282–287.

Lane, D. J. 1991. 16S／23S rRNA sequencing. In: (Stackebrandt, E. and Goodfellow, M. eds.) *Nucleic Acid Techniques in Bacterial Systematics*. New York: Wiley & Sons, pp. 115–175.

Lechner, S., Mayr, I. R., Francis, K. P., Pruo, B. M., Kaplan, T., WieOner-Gunkel, E., Gordon, S. A., Stewart, B. and Scherer, S. 1998. *Bacillus weihenstephanensis* sp. nov. is a new psychrotolerant species of the *Bacillus cereus* group. *Intl J System Bacteriol*. 48: 1373.

Lee, C. 1997. Lactic acid fermented foods and their benefits in Asia. *Food Control*. 8: 259-269.

Lee, J. W., J. G. Shin, E. H. Kim, H. E. Kang, I. B. Yim, J. Y. Kim, H. G. Joo, and H. J. Woo. 2004. Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum. J Veterinary Sci.* 5: 41–48.

Lee, W. J. and Lucey, J. A. 2004. Structure and Physical Properties of Yogurt Gels: Effect of Inoculation Rate and Incubation Temperature. *J Diary Sci.* 87(10): 3153-3164.

Lee, S. Y. and Kang, D. H. 2004. Microbial safety of pickled fruits and vegetables and hurdle technology. *Intl J Food Safety.* 4: 21-32.

Lee, H. R. and J. M. Lee. 2009. Anti-stress effects of kimchi. *Food Sci Biotech.* 18(1): 25–30.

Lee, J. H., Nam, S. H., Seo, W. T., Yun, H. D., Hong, S. Y., Kim, M. K. and Cho, K. M. 2012. The production of surfactin during the fermentation of cheonggukjang by potential probiotic Bacillus subtilis CSY191 and the resultant growth suppression of MCF-7 human breast cancer cells. *Food Chem.* 131(4): 1347-1354.

Leroy, F. and De Vuyst, L. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trend Food Sci Technol.* 15: 67–78.

Li, H. D., Hong, H. A., Barbosa, T. M., Henriques A. O. and Cutting, S. M. 2004. Characterization of *Bacillus* probiotics available for human use. *J Appl Mirobiol*. 70: 2161-2171.

Li, K., Zheng, T., Tian, Y., Yuan, J., Hang Z. and Hong, H. 2007. Beneficial effects of *Bacillus licheniformis* on intestinal microflora and immunity of the white shrimp, Litopenaeus *Vannarmee*. *Biotech Lett*. 22: 525-530.

Liem, I. T. H., Steinkraus, K. H. and Cronk, T. C. 1977. Production of vitamin B12 in tempeh, a fermented soybean food. *Appl Environ Microbiol*. 34: 773-776.

Lindgren, S. E. and Dobrogosz, W. J. 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. *FEMS Microbiol*. 87(1-2): 149-163. DOI: org/10.1016/0378-1097(90)90703-S.

Lopetcharat, K., Choi, Y. J., Park, J. W. and Daeschel, M. A. 2001. Fish sauce products and manufacturing: A review. *Food Rev Intl*. 17: 65–88.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem*. 193: 265-275.

Lücke, F. K. 1985. Fermented sausages. In: (Wood, B. J. B. ed.) *Microbiology of Fermented Foods,* Vol. 2. London: Elsevier Applied Science Publishers, pp: 41–83.

Luh, B. S. 1995. Industrial production of soy sauce. *J Indus Microbiol*. 14(6): 467-471.

Mahendra, D. S., Kannan, K. P. and Ramachandran, N. 2003. Towards Food Secure India (ed). Delhi: IHD-CESS-Manohar, pp-464.

Majumdar, R. K. and Basu, S. 2010. Characterization of the traditional fermented fish product *Lonailish* of North east India. *Indian J Trad Knowl*. 9(3): 453-458.

Mao, A. A. and Odyuo, N. 2007. Traditional fermented foods of the Naga tribes of North eastern, India. *Indian J Trad Knowl*. 6(1): 37-41.

Mao, A. A., Hyuniewta, T. M. and Sanjappa, M. 2008. Plant wealth of Noetheast India with reference to ethnobotany. *Indian J Trad Knowl*. 8(1): 96-103.

Maynard, A.J. 1970. Methods in food analysis. Academic Press New York, London, pp. 176.

Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligne´, B., Ganzle, M., Kort, R., Pasin, G., Pihlanto, A., Smid, E. J. and Hutkins, R. 2017. Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotech*. 44: 94–102.

Mathias, K., Ismail, B., Corvalan, C. M. and Hayes, K. D. 2006. Heat and pH Effects on the Conjugated Forms of Genistin and Daidzin Isoflavones. *J Agric Food Chem*. 54: 7495−7502.

Mayo, B., Ammor, M. S., Deldado, S. and Alegria, A. 2010. Fermented Milk Products. In: (Tamang, J.P. and Kailasapathy, K., eds.) *Fermented Foods and Beverages of the World*. CRC Press, pp: 263-288.

McKay, M., Buglass, A. J. and Lee, C. G. 2011. Fermented beverages: Beers, ciders, wines and related drinks. *Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects*, 1: 63–229.

McGovern, P. E., Zhang, J., Tang, J., Zhang, Z., Hall, G. R., Moreau, R. A., Nuñez, A., Butrym, E. D., Richards, M. P., Wang, C. S. and Cheng, G. 2004. Fermented beverages of pre-and proto-historic China. In: *Proceedings of the National Academy of Sciences of the United States of America*, 101(51): 17593-17598.

Meerak, J., Yukphan, P., Miyashita, M., Sato, H., Nakagawa, Y. and Tahara, Y. 2008. Phylogeny of γ-polyglutamic acid-producing *Bacillus* strains isolated from a fermented locust bean product manufactured in West Africa. *J General Appl Microbiol.* 54: 159–166.

Mehta, M. B., Eldin-Kamal, A. and Iwanski, Z. R. 2012. *Fermentation Effects on Food Properties. Chemical and Functional Properties of Food Components Series*. Taylor & Francis Group, CRC Press, Boca Raton, pp-399.

Miller, G.L. 1959. Use of Dinitrosalicyclic acid reagent for determination of reducing sugar. *Analytical Chem*. 31: 426-428.

Minamiyama, Y. and Okada, S. 2003. Miso: production, properties, and benefits to health. In: (Farnworth, E.R., ed.) *Handbook of Fermented Functional Foods*. CRC Press, Boca Raton, Fla, pp: 277–286.

Mishra, S., Ray, R. C., Panda, S. K. and Montet, D. 2017. Technological innovations in processing of fermented foods: an overview. In: (Ray, R. C. and Montet, D., eds.) *Fermented Foods-Part II: Technological Interventions*, CRC Press, Boca Raton, New York, pp 21-45.

Mohania, D., Kansal, V. K., Kruzliak, P. and Kumari, A. 2014. Probiotic dahi containing Lactobacillus acidophilus and Bifidobacterium bifidum modulates the formation of aberrant crypt foci, mucin-depleted foci, and cell proliferation on 1, 2-dimethylhydrazine-induced colorectal carcinogenesis in Wistar rats. *Rejuvenation Res*. 17(4): 325-333.

Mohite, B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *J Soil Sci Plant Nutr*. 13(3): 638-649.

Moktan, B., Saha, J. and Sarkar, P. K. 2008. Antioxidant activities of soybean as affected by *Bacillus*-fermentation to kinema. *Food Res Intl*. 41: 586-593.

Moore, E., Arnscheidt, A., Kruger, A., Strompl, C. and Mau, M. 1999. Simplified protocols for the preparation of genomic DNA from bacterial cultures. In: (Akkermans, A. D. L., Van Elsas, J. D. and de Bruijn, F. J., 2nd eds) *Molecular Microbial Ecology Manual*. Kluwer Academic Press, pp: 10-13.

Montet, D., Loiseau, G. and Zakhia-Rozis, N. 2006. Microbial technology of fermented vegetables. *In*: (Ray, R. C. and Ward, O.P., eds.) *Microbial Biotechnology in Horticulture.* Science Publishers, Enfield, NH, USA, pp 309–343.

Montet, D., Loiseau, G., Zakhia, N. and Mouquet, C. 1999. Fermented fruits and vegetables. In: (Joshi V. K. and Pandey, A., Vol. II, eds) *Biotechnology: Food Fermentation, Educational Publishers*. New Delhi, pp: 950 - 958.

Motarjemi, Y., Käferstein, F., Moy, G. and Quevedo, F. 1993. Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. *Bull World Health Org.* 71(1): 79.

Motarjemi, Y. 2002. Impact of small scale fermentation technology on food safety in developing countries. *Intl J Food Microbiol.* 75:213-229.

Mukherjee, R., Chakraborty, R. and Dutta, A. 2015. Role of fermentation in improving nutritional quality of soybean meal-a review. *Asian Aus J Animal Sci*, 29(11): 1523–1529. DOI: 10.5713/ajas.15.0627.

Muller, W. S., Allen, A. L., Sikes, A., Racicot, K. and Senecal, A. 2005. *Development of fermented taro as a food preservative ingredient in intermediate moisture products* (No. NATICK/TR-06/005). Army Natick Soldier Center Ma.

Müller, O. and Krawinkel, M. 2005. Malnutrition and health in developing countries. *CMAJ*. 173 (3): 279-286. DOI: 10.1503/cmaj.050342.

Mulaw, G. and Tesfaye, A. 2017. Technology and microbiology of traditionally fermented food and beverage products of Ethiopia: A review. *Afr J Microbio Res.* 11(21): 825-844. DOI: 10.5897/AJMR2017.8524.

Munimbazi, C. and Bullerman, L. B. 1998. Isolation and partial characterization of antifungal metabolites of *Bacillus pumilus*. *J Appl Microbiol*. 84: 959-968.

Muyzer, G., de Waal, E. C. and Uitterlinden, A. G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Env Microbiol*. 59(3): 695-700.

Muyzer, G. and Smalla, K. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek*. 73(1): 127-141.

Nagai, T. and Tamang, J. P. 2010. Fermented Legumes: Soybean and Non-Soybean Products. In: (Tamang, J.P. and Kailasapathy, K., eds.) *Fermented Foods and Beverages of the World*. CRC Press, Taylor & Francis Group, New York, pp: 191-224.

Nah, S. and Chau, C. 2010. Issues and challenges in defeating world hunger. *Trend Food Sci Technol*. 21: 544-557.

Nair, B. M. and Prajapati, J. B. 2003. The History of Fermented foods. In: (Fernworth, E.R., 2nd ed.) *Handbook of Fermented Functional Foods*. CRC Press, Boca Raton, London, NY, pp:1-24.

Narzary, Y., Brahma, J., Brahma, C. and Das, S. 2016. A study on indigenous fermented foods and beverages of Kokrajhar, Assam, India. *J Ethnic Foods*. 3: 284-291.

Nicoli, M. C., Anese, M. and Parpinel, M. 1999. Infuence of processing on the antioxidant properties of fruit and vegetables. *Trend Food Sci Technol*. 10: 94-100.

Nguyen, D. T. L., Van Hoorde, K., Cnockaert, M., De Brandt, E., Aerts, M. and Vandamme, P. 2013. A description of the lactic acid bacteria microbiota associated with the production of traditional fermented vegetables in Vietnam. *Intl J Food Microbiol*. 163(1): 19-27.

Nongdam, P. and Tikendra, L. 2014. The nutritional facts of bamboo shoots and their usage as important traditional foods of Northeast India. *Intl Scholarly Res Notices*. Article ID 679073. 1-17. DOI: org/10.1155/2014/679073.

Nout, M. J. R. and Ngoddy, P. O. 1997. Technological aspects of preparing affordable fermented complementary foods. *Food Control*. 8: 279–287.

Nout, M. J. R. and Motarjemi, Y. 1997. Assessment of fermentation as a household technology for improving food safety: A joint FAO / WHO workshop. *Food Control*. 8: 221-226.

Nout, M. J. R. 2001. Fermented foods and their production.In: (Adams, M.R. and Nout, M.J.R., eds.). *Fermentation and Food Safety*. Aspen Publishers, Inc., Gaitherssburg, MD, pp. 1–30.

Nout, M. J. R. and Kiers, J. L. 2005. Tempeh fermentation, innovation and functionality: Update into the third millenium. *J Appl Microbiol.* 98: 789–805.

Ntushelo, K. 2013. Identifying bacteria and studying bacterial diversity using the 16S ribosomal RNA gene-based sequencing techniques: A review. *Afr J Microbiol*. 7(49): 5533-5540.

Nuraida, L. 2015. A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. *Food Sci Human Wellness*. 4(2): 47-55.

Nyanzi, R. and Jooste, P. J. 2012. Cereal-based functional foods. In: (Rigobelo, E.C., eds). *Probiotics*. In Tech, pp-654. DOI: 10.5772/3444.

Odunfa, S. A. and Oyeyiola, G. F. 1985. Microbiological study of the fermentation of «ugba», a Nigerian indigenous fermented food flavour. *J Pl Foods.* 6(3): 155-163.

Ogbadu, L. J., Okagbue, R. N. and Ahmad, A. A. 1990. Glutamic acid production by Bacillus isolates from Nigerian fermented vegetable proteins. *World J Microbiol Biotech*. 6(4): 377-382.

Oguntoyinbo, F. A., Fusco, V., Cho, G., Kabisch, J., Neve, H., Bockelmann, W., Huch, M., Frommherz, L., Trierweiler, B., Becker, B., Benomar, N., Galvez, A., Abriouel, H., Omafuvbe, B. O., Shonukan, O. O. and Abiose, S. H. 2000. Microbiological and biochemical, changes in the traditional fermentation of soybean for `soy-daddawa' a Nigerian food condiment. *Food Microbiol*. 17: 469-474. DOI: 10.1006/fmic.1999.0332.

Oguntoyinbo, F. A., Sanni, A. I., Franz, C. M. A. P. and Holzapfel, W. H. 2007. *In vitro* fermentation studies for selection and evaluation of Bacillus strains as starter cultures for the production of okpehe, a traditional African fermented condiment. *Intl J Food Microbiol*. 113: 208-218.

Oguntoyinbo, F. A., Fusca, V., Cho, G., Kabisch, J., Neve, H., Bockelmann, W., Huch, M., Frommherz, L., Trierweiler, B., Becker, B., Benomar, N., Galvez, A., Abriovel, H., Holzapfel, W. H. and Franz, M. A. P. 2016. Produce from Africa’s gardens:

potential for leafy vegetables and fruit fermentations. *Frontiers Microbiol*. 7(981): 1-14. DOI: 10.3389/fmicb.2016.00981.

Oh, S-K,. Tsukamoto, C., Kim, K-W. and Choi, M-R. 2017. Investigation of glucosinolates, and the antioxidant activity of Dolsan leaf mustard kimchi extract using HPLC and LC-PDA-MS/MS. *J Food Chem*. 41(3): 1-10. DOI: 10.1111/jfbc.12366.

Ohara, H. and Yahata, M. 1996. L-Lactic acid production by *Bacillus* sp. in anaerobic and aerobic Culture. *J Fermen Bioeng.* 81(3): 272-174.

Olympia, M., Ono, H., Shinmyo, A. and Takano, M. 1992. Lactic acid bacteria in fermented fishery product, "burong bangus". *J Fermen Bioeng*. 73(3): 193-197.

Omodara, T. R. and Olowomofe, T. O. 2015. Effects of fermentation on the nutritional quality of African locust bean and soybean. *Intl Sci Res*. 4(1): 1069-1071.

Orel, R. and Trop, T. K. 2014. Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease. *World J Gastroenterol.* 20(33): 11505-11524.

Ouoba, L. I. I., Diawara, B., Jespersen, L. and Jakobsen, M. 2007. Antimicrobial activity of *Bacillus subtilis* and *Bacillus pumilus* during the fermentation of African locust bean (*Parkia biglobosa*) for Soumbala production. *J. Appl Microbiol*. 102: 963-970.

Ouoba, L. I., Nyanga-Koumou, C. A., Parkouda, C., Sawadogo, H., Kobawila, S. C., Keleke, S., Diawara, B., Louembe, D. and Sutherland, J. P. 2010. Genotypic diversity of lactic acid bacteria isolated from African traditional alkaline-fermented foods. *J Appl Microbiol.* 108: 2019–2029.

Oyewole, O. B. 1997. Lactic fermented foods in Africa and their benefits. *Food Control*. 8: 289-297.

Padmaja, G. and George, M. 1999. Oriental fermented foods. In: (Joshi, V. K. and Pandey, A., Vol. 2, eds.) *Biotechnology: Food Fermentation.* Educational Publishers and Distributors, New Delhi, pp. 523–581.

Papavergou, E. J. 2011. Biogenic amine levels in dry fermented sausages produced and sold in Greece. *Precedia Food Science*. 1: 1126-1131.

Paramithiotis, S., Hondrodimou, O. L. and Drosinos, E. H. 2010. Development of the microbial community during spontaneous cauliflower fermentation. *Food Res Intl*. 43(4): 1098–1103.

Paredes López, O., Harry, G. I. and Murray, E. D. 1988. Food biotechnology review: Traditional solid□state fermentations of plant raw materials — application, nutritional significance, and future prospects. *Crit Rev Food Sci Nutr* . 27(3): 159-187.

Park, M. W. and Park, Y. K. 1998. Changes of physicochemical and sensory characteristics of Oiji (Korean pickled cucumbers) prepared with different salts. *J Korean Soc Food Sci Nutr*. *27*: 419-424.

Park, K. Y., Jeong, J. K., Lee, Y. E. and Daily III, J. W. 2014. Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. *J Med Food*. *17*(1): 6-20.

Parkouda, C., Nielsen, D. S., Azokpota, P., Ouoba, L. II., Amoa-Awua, W. K., Thorsen, L., Hounhouigan, J. D., Jensen, J. S., Tano-Debrah, K., Diawara, B. and Jakobsen, M. 2009. The microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa and Asia. *Critical Reviews Microbiol*. 35(2): 139-156.

Patel, V. J., Tendular, S. R. and Chattoo, B. B. 2004. Bioprocess development for the production of an antifungal molecule by *Bacillus lichenifovmis* BC 98. *J Biosci Bioeng*. 98: 231-235.

Pederson, C. S. 1960. Sauerkraut. In: (Chichester, C. O., Mark, E. M., and Stewart, G. F., Vol. 10, eds.). *Advances in Food Research*. Academic Press, New York, NY, pp: 233–260.

Petchkongkaew, A. 2007. Reduction of mycotoxin contaminationlevelduring soybean: A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Technology, Suranaree University of Technology/Academic Year.

Pederson, C. S., and Ward, W. 1949. The effect of salt upon the bacteriological and chemical changes in fermenting cucumbers. New York State Agr. *Expt Sta Tech Bull. 169*: 287-290.

Perricone, M., Arace, E., Calo, G. and Sinigaglia, M. 2017. Ethnic fermented foods. In, Ellaiah, P., Adinarayana, K., Bhavani, Y., Padmaja, P. and Srinivasulu, B. 2002.

Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated Aspergillus species. *Process Biochem*. 38: 615-620.

Pitt, J. I. and Hocking, A. D. 2009. Fungi and Food spoilage (3rd Ed.). Springer Dordrecht Heidelberg London New York.

Pisoschi, A. M. and Negulescu, G. P. 2011. Methods for Total Antioxidant Activity Determination: A Review. *Biochem Anal Biochem*.1:1-10.

Prajapati, J. B. and Nair, B. M. 2003. The history of fermented foods. In: (Farnworth, R., ed.) *Handbook of Fermented Functional Foods.* New York, CRC Press, pp: 1-25.

Premarani, T. and Chhetry, G. K. N. 2011. Nutritional analysis of fermented soybean (*Hawaijar*). *J Sci Technol: Biol Environ Sci.* 7(1): 96-100.

Premarani, T. and Chhetry, G. K. N. 2011. Comparative biochemical analyses of soybean seeds, hawaijar and stored hawaijar. *J Mycopathological Res*. 49(1): 77-81.

Quoba, I., Thorsen, L. and Vamam, A. H. 2008. Enterotoxins and emetic toxins production by *Bacillus cereus* and other species of *Bacillus* isolated from Soumbala and Bikalga, African alkaline fermented food condiments. *Intl J Food Microbiol*. 124(3): 224-230. DOI: org/10.1016/j.ijfoodmicro.2008.03.026.

Rai, A. K., Palni, U. and Tamang, J. P. 2009. Traditional knowledge of the Himalayan people on production of indigenous meat products. *Indian J Trad Knowl.* 8 (1): 104–109.

Rai, A. K., Palni, U. and Tamang, J. P. 2009. Traditional knowledge of the Himalayan people on production of indigenous meat products. *Indian J Trad Knowl.* 8(1): 104–109.

Rao, R. V., Matthews, P., Eyzaguirre, P. B. and Hunter, D.. 2010. The global diversity of C. esculenta: ethnobotany and conservation. Biodiversity International, Rome.

Reddy, N. R., Sathe, S. K., Pierson, M. D. and Salunkhe, D. K. 1981. *Idli*, an Indian fermented food: A review. *J Food Quality*. 5: 89–101.

Rodríguez, R., Jime´nez, A., Fernandez-Bolanos, J., Guillen, R. and Heredia, A. 2006. Dietary fibre from vegetable products as source of functional ingredients. *Trend Food Sci Tech*. 17: 3–15.

Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., de Felipe, F. L., Gómez-Cordovés, C., José Mancheño, J.M. and Muñoz, R. 2009. Food phenolics and lactic acid bacteria. *Intl J Food Microbiol*. 132: 79–90.

Rolle, R. and Satin, M. 2002. Basic requirements for the transfer of fermentation technologies to developing countries. *Intl J Food Microbiol.* 75(3): 181–187.

Romero-Luna, H. E., Hernández-Sánchez, H. and Dávila-Ortiz, G. 2017. Traditional fermented beverages from Mexico as a potential probiotic source. *Annals Microbiol*. 67(9): 577-586.

Ross, R. P., Morgan, S. and Hill, C. 2002. Preservation and fermentation: Past, present and future. *Intl J Food Microbiol*. 79: 3–16.

Sahreen, S., Khan, M. and Khan, R. A. 2010. Evaluation of antioxidant activities of various solvent extracts of Carisa apaca fruits. *Food Chem*. 122: 1205-1211.

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

Sanjukta, S., Rai, A. K., Muhammed, A., Jeyaram, K. and Talukdar, N. C. 2015. Enhancement of antioxidant properties of two soybean varieties of Sikkim Himalayan region by proteolytic *Bacillus subtilis* fermentation. *J Functional Food*. 14: 650-658.

Sanjukta, S. and Rai, A. K. 2016. Production of bioactive peptides during soybean fermentation and their potential health benefits. *Trend Food Sci Tech*. 50: 1-10.

Sanjukta, S., Rai, A. K. and Sahoo, D. 2017. Bioactive molecules in fermented soybean products and their potential health benefits. In: (Ray, R. C. and Montet, D., eds.) *Fermented Foods, Part II: Technological Interventions*. CRC Press, Bacon Raton, New York.

Samruan, W., Oonsivilai, A. and Oonsivilai, R. 2012. Soybean and fermented soybean extract antioxidant activities. *Intl Scholarly Sci Res Inno*. 6(12): 1134-1137.

Sarangthem, K. and Singh, Th. N. 2003. Microbial bioconversion of metabolites from fermented succulent bamboo shoots into phytosterols. *Curr Sci*. 84(12): 1544-1547

Sarkar., P. K., Cook, P. E. and Owens, J. D. 1993. *Bacillus* fermentation of soybeans. *World J Microbiol Biotech*. 9(3): 295-299.

Sarkar, P. K., Tamang, J. P., Cook, P. E. and Owens, J. D. 1994. Kinema- a traditional soybean fermented food: Proximate composition and microflora. *Food Microbiol*. 11: 47–55.

Sarkar, P. K. and Tamang, J. P. 1995. Changes in the microbial profile and proximate composition during natural and controlled fermentations of soybeans to produce kinema. *Food Microbiol*. 12: 317–325

Sarkar, P. K., Jones, L. J., Craven, G. S., Somerset, S. M. and Palmer, C. 1997. Amino acid profiles of *kinema*, a soybean-fermented food. *Food Chem.* 59 (1): 69–75.

Sarkar, P. K., Morrison, E., Tingii, U., Somerset, S. M. and Craven, G. S. 1998. B-group Vitamin and mineral contents of soybeans during kinema production. *J Sci Food Agri.* 78: 498–502.

Sarkar, P. K. and Nout, M. J. R. (eds.). 2014. *Handbook of Indigenous Foods Involving Alkaline Fermentation*. CRC Press, Boca Raton, FL.

Sarojnalini, C. and Vishwanath, W. 1988. Studies on the biochemical composition of some freshwater fishes of Manipur. *J Adv Zool*. 9:1–5.

Sathe, G. and Mandal, S. 2016. Fermented products of India and its implications:A review. *Asian J Diary Food Res*. 35(1): 1-9.

Satya, S., Singhal, P., Bal, L. M. and Sudhakar, P. 2012. Bamboo shoot: a potential source of food security. *Mediterr J Nutr Metabol*. 5: 1-10.

Savadogo, A., Tapi, A., Chollet, M., Wathelet, B., Traoré, A.S. and Jacques, P. 2011. Identification of surfactin producing strains in *Soumbala* and *Bikalga* fermented condiments using polymerase chain reaction and matrix assisted laser desorption/ionization-mass spectrometry methods. *Intl J Food Microbiol.* 151: 299–306.

Schnurer, J. and Magnusson, J. 2005 Antifungal lactic acid bacteria as biopreservatives. *Trend Food Sci Technol*. 16(1): 70–78.

Seed, B., Lang, T., Caraher, M. and Ostry, A. 2013. Integrating food security into public health and provincial government departments in British Columbia, Canada. *Agriculture and Human Values.* 30(3): 457-470.

Sekar. S, and Mariappan. S. 2007. Usage of traditional fermented products by Indian rural floks and IPR. *Indian J Trad Knowl*. 6(1): 111-120.

Settanni, L. and Corsetti, A. 2008. Application of bacteriocins in vegetable food biopreservation. *Intl J Food Microbiol*. 121(2): 123–138.

Selhub, E. M., Logan, A. C. and Bested, A. C. 2014. Fermented foods, microbiota, and mental health: ancient practice meets nutritional psychiatry. *J Physiological Anthropol*. 33(2): 1-12.

Selvakumaran, S., Kapley , A., Kalia, V. C. and Purohit, H. J. 2008. Phenotypic and phylogenic groups to evaluate the diversity of Citrobacter isolates from activated biomass of effluent treatment plants. *Bioresource Technol*. 99: 1189–1195.

Selvendran, R. R. and Robertson, J. A. 1994. Dietary fibre in foods: amount and type. In: Amado, R. and Barry, J.L. (eds). Metabolic and physiological aspects of dietary fibre in food. *Commission of the European Communities*, Luxembourg, pp 11–20

Sharma, N., Handa, S. and Gupta, A. 2013. A comprehensive study of different traditional fermented foods/beverages of Himachal Pradesh to evaluate their nutrition impact on health and rich biodiversity of fermenting microorganisms. *Intl J Res Appl Natural Soc Sci.* 1(3): 19-28.

Shin, D. and Jeong, D. 2015. Korean traditional fermented soybean products: Jang. *J Ethnic Food. 2*(1): 2-7.

Shon, M., Lee, J., Choi, J., Choi, S., Nam, S., Seo, K., Lee, S., Sung, N. and Park, S. 2007. Antioxidant and free radical scavenging activity of methanol extract of chungkukjang. *J Food Comp Anal*. 20: 113–118.

Shin, D. and Jeong, D. 2015. Korean traditional fermented soybean products: Jang. *J Ethnic Foods*. 2: 2-7.

Shon, M., Lee, J., Choi, J., Choi, S., Nam, S., Seo, K., Lee, S., Sung, N, and Park, S. 2007. Antioxidant and free radical scavenging activity of methanol extract of *chungkukjang*. *J Food Comp Anal*. 20: 113-118.

Shin, D. and Jeong, D. 2015. Korean traditional fermented soybean products: Jang. *J Ethnic Foods*. *2*(1): 2-7.

Shahcheraghi, S. H., Ayatollahi, J. and Lotfi, M. 2015. Applications of *Bacillus subtilis* as an important bacterium in medical sciences and human life. *Tropical J Medical Res*. 18(1): 1-4.

Shekhawat, K., Rathore, T. S., Premi, O. P., Kandpal, B. K. and Chauhan, J. S. 2012. Advances in agronomic management of Indian mustard (*Brassica juncea* (L.) Czernj. Cosson): An overview. *Intl J Agronomy*, Article ID 408284, pp.1-14.

Shekhar, T. C. and Anju, G. 2014. Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. Leaves . *American J Ethnomed.* 1(4): 244-249.

Shrivastava, K., Greeshma, A. G. and Shrivastava, B. 2012. Biotechnology in Tradition-A process technology of alcoholic beverages practised by different tribes of Arunachal Pradesh, North East India. *Indian J Trad Knowl*. 11(1): 81-89.

Singh, P. K. and Singh, K. I. 2006. Traditional alcoholic beverage, *Yu* of *Meitei* communities of Manipur. *Indian J Trad Knowl.* 5(2): 184–190.

Singh, A., Singh, R. K. and Sureja, A. K. 2007. Cultural significance and diversities of ethnic food of Northeast India. *Indian J Trad Knowl*. 6(1): 79-94.

Singh, R. K., Singh, A. and Sureja, A. K. 2007a. Traditional foods of monpa tribe of West Kameng, Arunachal Pradesh. *Indian J Trad Knowl.* 6(1):25–36.

Singh, H. D., Singh, S. A., Singh, N. R. and Singh, R. 2011. Biochemical composition of Soibum-A fermented bamboo shoot and its dynamics during fermentation in real time model. In: Proceedings of 9th International Conference on Food Engineering and Biotechnology, Bangkok, Thailand. 7^{th}-9^{th}, May. 9: 11-18.

Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Ame J Enol Viticult*. 16: 144-158

Singh, A., Singh, R. K. and Sureja, A. K. 2007. Cultural significance and diversities of ethnic food of Northeast India. *Indian J Trad Knowl.* 6(1): 79-94.

Singh, V. P., Pathak, V. and Verma, A. K. 2012. Fermented meat products: organoleptic qualities and biogenic amines–a review. *Ame J Food Technol*, 7(5): 278-288.

Sohliya, I., Joshi, S. R., Bhagobaty, R. K. and Kumar, R. 2009. *Tungrymbai*-A traditional fermented soybean food of the ethnic tribes of Meghalaya. *Indian J Trad Knowl.* 8(4): 559-561.

Smith, I. F. and Eyzaguirre, P. 2007. African leafy vegetables: their role in the World Health Organization's global fruit and vegetable initiative. *Afr J Food, Agri Nutr Dev.* 7(3&4): 1-8.

Sonar, N. R., Vijayendra. S. V., Prakash, M., Saikia, M., Tamang, J. P. and Halami, P. M. 2015. Nutritional and functional profile of traditional fermented bamboo shoot based products from Arunachal Pradesh and Manipur states of India. *Intl Food Res J*. 22 (2): 788-797.

Sonar, N. R., Vijayendra, S. V. N., Prakash, M., Saikia, M., Tamang, J. P.and Halami, P. M. 2015. Nutritional and functional profile of traditional fermented bamboo shoot based products from Arunachal Pradesh and Manipur states of India. *Intl Food Res J*. 22(2): 788-797

Soni, S. K. and Sandhu, D. K. 1990. Indian fermented foods: Microbiological and Biochemical aspects. *Indian J Microbiol.* 30(2): 135–137.

Soni, S. K. and Sandhu, D. K. 1999. Fermented cereal products: In (Joshi, V. K. and Pandey, A., Vol.2. eds.) *Biotechnology: Food Fermentation*. Educational Publishers, Ernakulam, India, pp: 895-949.

Soukoulis, C., Panaqietidis, P., Koureli, R. and Tzia, C. 2007. Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality. *J Diary Sci*. 90(6): 2641-2654.

Speranza, B., Bevilacqua, A., Corbo, R. M. and Sinigaglia, M. 2017. Starter Cultures in food production. Wiley Blackwell, pp: 384-406.

Steinkraus, K. H., Hwa, Y. B., Van Buren, J. P., Providenti, M. I. and Hand, D. B. 1960. Studies on tempeh—an Indonesian fermented soybean food. *Food Res.* 25:777–788.

Steinkraus, K. H. 1979. Nutritionally significant indigenous foods involving an alcoholic fermentation. In: (Gastineau, C. F., Darby, W. J. and Turner, T. B., eds.) *Fermented Food Beverages in Nutrition*. Academic Press, London, pp. 35–59.

Steinkraus, K.H. 1983. Fermented foods, feeds and beverages. *Biotech Adv.* 1: 31–46.

Steinkraus, K. H. 1996. *Handbook of Indigenous Fermented Food* (2nd ed.) Marcel Dekker, Inc., New York, NY.

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

Steinkraus, K. H. 1998. Bio-enrichment: production of vitamins in fermented foods. In: (Wood, B.J.B. 2nd ed) *Microbiology of Fermented Foods,* Blackie Acedamic and Professional, pp: 603-621.

Steinkraus, K. H. 2002. Fermentations in world food processing. *Compr Rev Food Sci Food Safety*. 1: 23-32.

Stackebrandt, E., Breymann, S., Steiner, U., Prauser, H., Weiss, N. and Schumann, P. 2002. Re-evaluation of the status of the genus *Oerskovia*, reclassification of *Promicromonospora enterophila* (Jáger *et al.* 1983) as *Oerskovia enterophila* comb. nov. and description of *Oerskovia jenensis* sp. nov. and *Oerskovia paurometabola* sp. nov. *Intl J Systematic and Evol Microbiol*. 52: 1105-1111.

Stanton, C., Ross, R. P., Fitzgerald, G. F. and Sinderen, D. V. 2005. Fermented functional foods based on probiotics and their biogenic metabolites. *Curr Opin Biotech*. 16: 198–203

Sundhagul, M., Smanmathuroj, P. and Bhodacharoen, W. 1972. Thua-nao: a fermented soybean food of northern Thailand. I. traditional processing method. *Thai J Agricult Sci*. 5: 43-56.

Sweeney, M. J. and Dobson, A. D. W. 1998. Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *Intl J Food Microbiol.* 43(3): 141–158.

Swain, M. R., Anandharaj, M., Ray, R. C. and Rani, R. P. 2014. Fermented fruits and vegetables of Asia: A potential source of probiotics**.** *Biotech Res Intl*. Article ID 250424, pp. 1-19. DOI: org/10.1155/2014/250424.

Tanti, B., Gurung, L., Sarma, H. K. and Buuragohain, A. K. 2010. Ethnobotany of starter cultures used in alcohol fermentation by a few ethnic tribes of Northeast India. *Indian J Trad Knowl.* 9(3): 463-466.

Tamang, J. P. and Sarkar, P. K. 1988. Traditional fermented foods and beverages of Darjeeling and Sikkim—A review. *J Sci Food Agrculti.* 44(4): 375–385.

Tamang, J. P. and Sarkar, P. K. 1993. Sinki—A traditional lactic acid fermented radish tap root product. *J General Appl Microbiol.* 39: 395–408.

Tamang, J. P. and Nikkuni, S. 1996. Selection of starter culture for production of kinema, fermented soybean food of the Himalaya. *World J Microbiol Biotech.* 12: 629–635.

Tamang, J. P. and Sarkar, P. K. 1996. Microbiology of mesu, a traditional fermented bamboo shoot product. *Intl J Food Microbiol*. 29:49-58.

Tamng, J. P. 1998. Role of Microorganisms in Traditional Fermented Foods. *Indian Food Industry*. 17(3): 162-166.

Tamang, J. P. and Nikkuri, S. 1998. Effect of temperatures during pure culture fermentation of Kinema. *World J Microbiol Biotech.* 14: 847–850.

Tamang, J. P. and Holzapfel, W. H. 1999. Biochemical identification techniques-modern techniques: Microfloras of fermented foods. In: (Robinson, R. K., Batt, C. A. and Patel, P. D., eds). *Encyclopedia of Food Microbiology.* Academic Press, London, pp: 249–252.

Tamang, J. P. 2001. Food culture in the Eastrern Himalayas. *J Himalayan Res Cult Foundation*. 5(3–4): 107–118.

Tamang, J. P., Thapa, S., Dewan, S., Jojimab ,Y., Fudou, R. and Yamanaka, S. 2002. Phylogenetic analysis of *Bacillus* strains isolated from fermented soybean foods of Asia: Kinema, Chungkokjang and Natto. *J Hill Res*. 15(2): 56-62.

Tamang, J. P. 2003. Native microorganisms in the fermentation of Kinema. *Indian J Microbiol.* 43(2): 127-130.

Tamang, J. P., Tamang, B., Schillinger, U., Franz, C. M. A. P., Gores, M. and Holzapfel, W. H. 2005. Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. *Intl J Food Microbiol.* 105: 347-356.

Tamang, J. P. and Thapa, S. 2006. Fermentation dynamics during production of bhaati jaanr, a traditional fermented rice beverage of the Eastern Himalayas. *Food Biotech.* 20: 251–261.

Tamang, B., Tamang, J. P., Schillinger, U., Franz, C. M. A. P., Gores, M. and Holzapfel, W. H. 2008. Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented tender bamboo shoots of North East India. *Intl J Food Microbiol.* 121: 35–40.

Tamang, J. P. 2009. The Himalayas and Food Culture. In: (Tamang, J. P., ed.) *Himalayan Fermented Foods: Microbiology, Nutrition and Ethnic Values.* CRC Press Taylor & Francis, Boca Raton, FL, pp. 1–23.

Tamang, J. P. 2009a. Fermented milks. In: (Tamang, J. P., ed.) *Himalayan Fermented Foods: Microbiology, Nutrition and Ethnic Values.* CRC Press Taylor & Francis, Boca Raton, FL, pp. 216–224.

Tamang, J. P. 2009b. Ethnic Fish Prodcuts. In: (Tamang, J. P., ed.) *Himalayan Fermented Foods: Microbiology, Nutrition and Ethnic Values.* CRC Press Taylor & Francis, Boca Raton, FL, pp: 139-158.

Tamang, J. P. 2009c. Fermented Vegetables. In: (Tamang, J. P., ed.) *Himalayan Fermented Foods: Microbiology, Nutrition and Ethnic Values.* CRC Press Taylor & Francis, Boca Raton, FL, pp: 25-64.

Tamang, J. P. 2009d. Ethnic meat products. In: (Tamang, J. P., ed.) *Himalayan Fermented Foods: Microbiology, Nutrition and Ethnic Values.* CRC Press Taylor & Francis, Boca Raton, FL, pp. 161–185.

Tamang, B. and Tamang, J. P. 2009a. Traditional knowledge of bioperservation of perishable vegetable and bamboo shoots in Northeast India as food resources. *Indian J Trad Knowl*. 8(1): 89-95.

Tamang, B. and Tamang, J. P. 2009b. Lactic acid bacteria isolated from indigenous fermented bamboo products of Arunachal Pradesh in India and their functionality. *Food Biotech*. 23: 133-147.

Tamang, J. P., Chettri, R. and Sharma, R. M. 2009. Indigenous knowledge of Northeast women on production of ethnic fermented soybean foods. *Indian J Trad Knowl*. 8(1): 122–126.

Tamang, J. P. and Samuel, D. 2010. Dietary cultures and antiquity of fermented foods and beverages. In: (Tamang JP and Kailasapathy K., eds.) *Fermented Foods and Beverages of the World*. CRC Press, Taylor & Francis, Boca Raton, FL, pp: 1–40.

Tamang, J. P. 2010. Diversity of fermented beverages and alcoholic drinks. In: (Tamang, J. P. and Kailasapathy, K., eds.) *Fermented Foods and Beverages of the World*. CRC Press, Taylor & Francis, Boca Raton, FL, pp. 85–125.

Tamang, J. P. 2011. Prospects of Asian Fermented Foods in Global Markets. In: *The 12th Asian Food Conference*. BITEC Bangna, Bangkok, Thailand.

Tamang, J. P. 2012. Ancient food culture: History and ethnicity. In: (Lama, M.P., ed.) *Globalisation and Cultural Practices in Mountain Areas: Dynamics, Dimensions and Implications.* Sikkim University Press and Indus Publishing Company, New Delhi, pp. 43–63.

Tamang, J. P., Tamang, N., Thapa, S., Dewar, S., Tamang, B., Yonzan, H., Rai, A. K., Chettri, R., Chakrabarty, J. and Kharel, N. 2012. Microorganisms and Nutritional value of Ethnic fermented foods and alcoholic beverages of North East, India. *Indian J Trad Knowl,* 11(1): 7-25.

Tamang, J. P., Tamang, N., Thapa, S., Dewar, S., Tamang, B., Yonzan, H., Rai, A. K., Chettri, R., Hasan, M. N., Sultan, M. Z. and Mar-E-Um, M. 2014. Significance of Fermented Food in Nutrition and Food Science. *J Scientific Res*. 6(2): 373-386.

Tamang, J. P. 2015. Naturally fermented ethnic soybean foods of India. *J Ethnic Foods*. 2: 8-17.

Tamang, J. P., Shin, D., Jung, S. and Chae, S. 2016a. Functional properties of microorganisms in fermented foods. *Frontiers Microbiol*. 7(578): 1-13.

Tamang, J. P., Watanabe, K. and Holzapfel, W. H. 2016b. Review: Diversity of Microorganisms in Global Fermented Foods and Beverages. *Frontiers Microbiol.* 7(377): 1-28.

Tamang, B. and Tamang, J. P. 2009. Traditional knowledge of biopreservation of perishable vegetable and bamboo shoots in Northeast India as food resources. *Indian J Trad Knowl*. 8(1): 89-95.

Tamime, A. Y. and Robinson, R. K. 2007. *Yoghurt Science and Technology*. Cambridge: Woodhead Publishing Ltd.

Tendulkar, S. R., Saikumari, Y. K., Patel, V. J., Raghotama, S., Munshi, T. K., Balaram, P. and Chattoo, B. B. 2008. Isolation, purification and characterization of an antifungal

molecule produced by *Bacillus licheniformis* BC 98 and its effect on phytopathogen *Mangaporthagrisea*. *J Appl Microbiol*. 103(6): 2331-2339.

Teramoto, Y., Yoshida, S. and Ueda, S. 2002. Characteristics of rice beer (*Zutho*) and a yeast isolated from the fermented product in Nagaland, India. *World J Microbiol Biotech.* 18: 813-816.

Thakur, K., Rajani, C. S., Tomar, S. K. and Panmei, A. 2016. Fermented bamboo shoots: a rich niche for beneficial microbes. *J Bacteriol Mycol*. 2(4): 1-8.

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

Thapa, N. 2002. Studies on microbial diversity associated with some fish products of the Eastern Himalayas. Ph.D. thesis, North Bengal University, Darjeeling.

Thapa, N., Pal, J. and Tamang, J. P. 2004. Microbial diversity in ngari, hentak and tungtap, fermented fish products of North-East India. *World J Microbiol Biotech.* 20: 599-607.

Thapa, N. and Tamang, J. P. 2015. Functionality and therapeutic values of fermented foods. In: (Tamang, J.P., eds) *Health Benefits of Fermented Foods and Beverages*. CRC Prss, Bacon Raton, NY, pp.111-168.

Thapa, N. 2016. Ethnic fermented and preserved fish products of India and Nepal. *J Ethnic Foods*. 3(1): 69-77.

Thokchom, S. and Joshi, S. R. 2012. Microbial and chemical changes during preparation in the traditionally fermented soybean product *Tungrymbai* of ethnic tribes of Meghalaya. *Indian J Trad Knowl*. 11(1): 139-142.

Tiwari, S. C. and Mahanta, D. 2007. Ethnological observations on fermented food products of certain tribes of Arunachal Pradesh. *Indian J Tradi Knowl*. 6(1): 106-110.

Toit, M. D., Franz, C. M. A. P., Dicks, L. M. T. and Holzapfel, W. H. 2000. Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. *J Appl Microbiol*. 88: 482-494.

Tolhurst, G., Heffron, H., Lam, Y.S., Parker, H. E., Habib, A. M., Diakogiannaki, E., Cameron, J., Grosse, J., Reimann, F. and Gribble, F. M. 2012. Short-chain fatty acids

stimulate glucagon-like peptide-1 secretion via the G-protein–coupled receptor FFAR2. *Diabetes*. 61(2): 364-371.

Tufa, M. A., Urga, K., Weledesemayat, G. T. and Mitiku, B. G. 2016. Development and Nutritional Assessment of Complementary Foods from Fermented Cereals and Soybean. *J Food Sci Nutr*. 2: 1-14. DOI: 10.24966/FSN-1076/100014.

Uchoi, D., Roy, D., Majumdar, R. K. and Debbarma, 2015. Diversified traditional cured food products of certain indigenous tribes of Tripura, India. *Indian J Tradi Knowl*. 14(3): 440-446.

Van Veen, A. G. 1953. Fish preservation in Southeast Asia. *Adv Food Res.* 4: 209–232

Van Veen, A. G. 1957. *Fermented Protein-Rich Foods*. FAO report no. FAO/57/3/1966.

Wacher, C., Díaz-Ruiz, G. and Tamang, J. P. 2010. Fermented Vegetable Products. In: (Tamang, J.P. and Kailasapathy, K., eds.) *Fermented Foods and Beverages of the World*. CRC Press, Taylor & Francis, Boca Raton, FL, pp. 149-190.

Waikhom, S. D., Bengyella, L., Roy, P. and Talukdar, N. C. 2015. Insights on predominant edible bamboo shoot proteins. *Afr J Biotech*. 14(7): 1511-1518.

Wang, H. L. and Hesseltine, G. W. 1979. *Prescott and Dunn's Industrial Microbiology Read J*. AVI Publishing Go. Inc. Westport, CT, pp. 492.

Wang, L. -J., Li, D., Zou, L., Chen, X. D., Cheng, Y, Q., Yamaki, K. and Li, L-T. 2007. Antioxidative activity of douchi (a Chinese traditional salt-fermented soybean food) extracts during its processing. *Intl J Food Properties*. 10: 1–12.

Wang, C-Y, Wu, S-J. and Shyu, Y-T. 2014. Antioxidant properties of certain cereals as affected by food-grade bacteria fermentation. *J Biosci Bioeng*. 117(4): 449-456.

Watanabe, K., Fujimoto, J., Tomii, Y., Sasamoto, M., Makino, H., Kudo, Y. and Okada, S. 2009. *Lactobacillus kisonensis* sp. nov., *Lactobacillus otakiensis* sp. nov., *Lactobacillus* rapi sp. nov. and *Lactobacillus sunkii* sp. nov., heterofermentative species isolated from sunki, a traditional Japanese pickle. *Intl J Systematic Evolutionary Microbiol.* 59: 754–760.

Woese, C. R., Stackebrandt, E., Macke, T. J. and Fox, G. E. 1985. A phylogenetic definition of the major *Eubacterial* taxa. *Systematic Appl Microbiol*. 6(2): 143-151.

Wood, B. J. B. 1994. Technology transfer and indigenous fermented foods. *Food Res Intl*. 27: 383-389.

Wu, J. and Ding, X. 2001. Hypotensive and physiological effect of angiotensin converting enzyme inhibitory peptides derived from soy protein on spontaneously hypertensive rats. *J Agri Food Chem*. 49(1): 501-506.

Wu, J., Zheng, J., Xia, X. and Kan, J. 2015. Purification and structural identification of polysaccharides from bamboo shoots (*Dendrocalamus latiflorus*). *Intl J Mol Sci*. 16(7): 15560-15577.

Yadav, H., Jain, S. and Sinha, P. R. 2007. Production of free fatty acids and conjugated linoleic acid in probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* during fermentation and storage. *Intl Diary J*. 17(8): 1006-1010.

Yasmine, M. 2000. impact of small scale fermentation technology on food safety in developing countries. *Intl J Food Microbiol*. 75(3): 213-229.

Yonzan, H. and Tamang, J. P. 2009. Traditional processing of *Selroti*—a cereal-based ethnic fermented food of the *Nepalis. Indian J Trad Knowl.* 8(1): 110–114.

Yadav, B. S., Yadav, R., Yadav. R. B. and Garg, M. 2016, Antioxidant activity of various extracts of selected gourd vegetables. *J Food Sci Technol*. 53:1823-1833.

Yang, J-H., Mau, J-L., Ko, P-T. and Huang, L-C. 2000. Antioxidant properties of fermented soybean broth. *Food Chem*. 71: 249-254

Yan, Z., Zheng, X. W., Chen, J. Y., Han, J. S. and Han, B. Z. 2013. Effect of different *Bacillus* strains on the profile of organic acids in a liquid culture of Daqu. *J Institute Brewing Distilling*. 119: 78-83.

Zhang, W., Xiao, S., Samaraweera, H., Lee, E. J. and Ahn, D. U. 2010. Improving functional value of meat products. *Meat Sci*. 86:15-31.

Appendix - I

Biochemical Analysis Reagents

Phosphate Buffer (0.1 M)

NaH_2PO_4	3.1 g
Na_2HPO_4	10.9 g
Distilled water	1000ml
*p*H	7

Lowry's Reagent

Reagent A

Na_2CO_3	20 g
NaOH	4 g
Distilled water	1000 ml

Reagent B

$CuSO_4$	10 g
$KNaC_4H_4O_6 \cdot 4H_2O$	10g
Distilled water	1000 ml

Reagent C- Folin-ciocalteau

$Na_2WO_4, 2H_2O$	100 g
$NaMoO_4, 2H_2O$	25 g
Distilled water	700 ml
H_3PO_4	50 ml
Li_2SO_4	150 g
Liquid bromine	4-5 drops

Contd...

Dinitrosalicyclic Acid Reagent (DNS Reagent)

DNS	1 g
Phenol	200 mg
Sodium Sulphite	50 mg
NaOH	1 g
Distilled water	100 ml

40% Rochelle salt solution

$KNaC_4H_4O_6$, $4H_2O$	40 g
Distilled water	100 ml

Appendix - II

Microbiology Reagents

Crystal violet

Crystal violet	10 g
Absolute alcohol	100 ml
Distilled water	900 ml

Dissolve the dye in the alcohol, filter and add water. Crystal violet is used at the concentrations of 0.5 – 2%.

Gram's iodine

Iodine	1 g
Potassium iodide	2 g
Distilled water	30 ml

Safranin

Safranin saturated alcohol solution

1. 2.5 g/ 100 ml of 95% alcohol - 10 ml
2. Water – 90 ml

3% Hydrogen Peroxide

H_2O_2	3 g
Distilled water	100 ml

Contd...

Microbiology Media

Nutrient Media

Nutrient Agar Medium

Peptone	5.0 g
Beef extract	3.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	1000 ml
*p*H	6.8

Tryptone Soya Agar

Pancreatic digest of casein	15.0 g
Digest of soybean meal	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	1000 ml
*p*H	7.3

MRS Agar

Peptone	10.0 g
Meat extract	10.0 g
Glucose	20.0 g
Yeast extract	5.0 g
Tween 80	1 ml
Dipotassium hydrogen phosphate	2.0 g
Sodium acetate	5.0 g
Triammonium citrate	2.0 g
Magnesium sulphate	0.2 g
Manganese sulphate	0.2g
Agar	15.0g
Distilled water	1000 ml
*p*H	6.2

Contd...

Plate Count Agar

Casein hydrolysate	5.0 g
Yeast extract	2.50 g
Dextrose	20 g
Agar	15.0 g
Distilled water	1000 ml
*p*H	7.0

Violet Red Bile Glucose Agar

Crystal violet	2 g
Agar	15.0 g
Distilled water	1000 ml
*p*H	7.4

Potato Dextrose Agar

Potato cubes	200.0 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 ml
*p*H	5.6

Yeast Malt Agar

Yeast extract	30 g
Peptone	5.0 g
Agar	20.0 g
Distilled water	1000 ml
*p*H	5.4

APPENDIX – III

Details of Microbes Isolated From Different Fermented Foods, Genbank Accession No. and Sequences of 16S rRNA Regions

Fermented Product: *Axone/Akhuni*

Isolate Name	Closest related microorganism	Genbank Accession No.	Sequences
BJ-DEBCR-1	***Staphylococcus epidermis***	**KU301333**	GAGGAGCTTGCTCCTCTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAA GACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATATTGAACCGCATGGTTCAATA GTGAAAGACGGTTTTGCTGTCACTTATAGATGGATCCGCGCCGCATTAGCTAGTTGGTAAGGTAA CGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGA CACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACG GAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAA TGTGTAAGTAACTATGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAG CAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGC GGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAAC TTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGA ACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCA AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCC GCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAA CTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCG AAGAACCTTACCAAATCTTGACATCCTCTGACCCCTCTAGAGATAGAGTTTTCCCCTTCGGGGGAC AGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGAC AAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTG CTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCT CAGTTCGGATTGTAGTCTGCAACTCGACTATATGAAGCTGGAATCGCTAGTAATCGTAGATCAGC ATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAAC ACCCGAAGCCGGTGGAGTAACCATTTGGAGCTAGCCGTCGAAGGTGGACAAATGA
BJ-DEBCR-2	***Bacillus licheniformis***	**KU301334**	ACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGAT GTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGA AACCGGGGCTAATACCGGATGCTTGTTTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTAGCT

			ACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACG ATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC GGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGT GATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCG GTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT AGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGAT GTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGG AGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGG CGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC CTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCA GCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGG TCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCCCCTTCGGGGGCAGAGTGACAGGTGGTG CATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGAT CTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACC GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACA ATGGGCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGTTCTCAGTT CGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATC
BJ-DEBCR-3	***Bacillus subtilis***	**KU301335**	GCCAAGACTCTAATACATGCTTGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCG GACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT AATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACA GATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCC GACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC AGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTT TTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGAC GGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA GCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCC CCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAA TTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTG GTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTC CACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCA TTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCG CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC CTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC AGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT CAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC

			GAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGA CTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGG
BJ-DEBCR-21	***Alcaligenes faecalis***	**KX364204**	ATGCTTTACACATGCAAGTCGAACGGCAGCACGAGAGAGCTTGCTCTCTTGGTGGCGAGTGGCGG ACGGGTGAGTAATATATCGGAACGTGCCCAGTAGCGGGGGATAACTACTCGAAAGAGTGGCTAA TACCGCATACGCCCTACGGGGGAAAGGGGGGGATTCTTCGGAACCTCTCACTATTGGAGCGGCCG ATATCGGATTAGCTAGTTGGTGGGGTAAAGGCTCACCAAGGCAACGATCCGTAGCTGGTTTGAGA GGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAA TTTTGGACAATGGGGGAAACCCTGATCCAGCCATCCCGCGTGTATGATGAAGGCCTTCGGGTTGT AAAGTACTTTTGGCAGAGAAGAAAAGGTATCTCCTAATACGAGATACTGCTGACGGTATCTGCAG AATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGG AATTACTGGGCGTAAAGCGTGTGTAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGGGCTCAA CCTTGGAACTGCATTTTTAACTGCCGAGCTAGAGTATGTCAGAGGGGGGTAGAATTCCACGTGTA GCAGTGAAATGCGTAGATATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGATAATACT GACGCTCAGACACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAA ACGATGTCAACTAGCTGTTGGGGCCGTTAGGCCTTAGTAGCGCAGCTAACGCGTGAAGTTGACCG CCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGG ATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGTCTGGAATGCCG AAGAGATTTGGCAGTGCTCGCAAGAGAACCGGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGTTGCTACGCAAGAG CACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGC CCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGGACAGAGGGTCGCCAACCCGCGAGGGG GAGCCAATCTCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGG AATCGCTAGTAATCGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCC CGTCACACCATGGGAG
BJ-DEBCR-22	***Bacillus cereus***	**KX364205**	GCGGCTACGCTGGCGACGTGCCTATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTATG AAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGG GAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGG CTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAA CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT ACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGA GTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGC TGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTG ATGTGAAAGCCCACGGCTCAA
BJ-DEBCR-24			GGCGGCGTGCTATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGG ACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTA ATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAG

	Bacillus subtilis	**MF487822**	ATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCG ACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTT CGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGG TACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC GTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCC CGGCTCAACTGGGGAGGGTCATTGGGAACTGGGGAACTTGAGTGCAGAAAAGGAGAGTGGAATT CCACGTGTAGCGGTGAAAATGCGTAGAGATGTGGAGGAACACC
BJ-DEBCR-29	***Bacillus cereus***	**MF487826**	AGCTTGGCGCGTGCTATAATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCG GCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGG GCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTT ATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTA GCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC AGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAG GCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAA CAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATCATTGGGCGTAAAGCGCGC GCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTG GGAGACTCGAGTG
BJ-DEBCR-33	***Bacillus licheniformis***	**MF487831**	TATAATGCAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTCAGCGGCGGACGGGTGAGTAA CACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTT GATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGG CGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGT GATCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTT CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAA ACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAG AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAAT TATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGG GGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCG GTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGAC GCTAAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGC CTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA GCATGTGGTTTAATTCGAAGCAACAAGA

Fermented Product: *Anishi*

Isolate Name	Closest related microorganism	Genbank Accession No.	Sequences
BJ-DEBCR-4	***Bacillus licheniformis***	**KU30136**	TGCAAGTCGAGCGGACCGACGGGAGCTTGCTCCCTTAGGTCAGCGGCGGACGGGTGAGTAACACG TGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTT GAACCGCATGGTTCAAACATAAAAGGTGGCTTTTCGCTACCACTTACAGATGGACCCGCGGCGCA TTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATC GGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTG TTGTTAGGGAAGAACAAGTACCGTTCGAACAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCC ACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGG GCGTAAAGCGCGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGG TCATTGGAAACTGGGGGAACTGAGTGCATAAGAGGAGAGTAGAATTCCACGTGTAGCGGTGAATG GCTTAGAGATGTGGATGAACACCAGT
BJ-DEBCR-6	***Bacillus subtilis***	**KU854954**	GGCTTNCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGAC GGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATA CCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATG GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACC TGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTA GGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGG ATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTAC CTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTG TCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCT CAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACG TGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAACGCGACTCTCTGGTCTGTA ACTGACTCTGAGGAGCGAAAGCGTGGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCC GTAAACGATGAGTGCTAAGTGTTAGGGGGGTTTCCGCCCCCTTAGTGCTGCAGCTAACGCATTAA GCACTCCGCCCTGGGGGAGTACGGTCGCCAAAGACTGAAACTCAAAGGAATTGACGGGGGCCCG CACAACCTTTTGAC
BJ-DEBCR-16	***Enterococcus faecalis***	**KU854962**	TAGTACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTA AGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCA GAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATAGAGGGAGGAACACCAGTG GCGAAGGCGGCTCTCTCGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATT AGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGT GCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGA ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA

			CCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGT GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT TATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAAG GTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAG TACAACGAGTCGCTAGACCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAGTTCGGATTGCAG GCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATA CGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGA GGTAACCTTTTGGAGCCAGCGCGGCTCAGGNGTACC
BJ-DEBCR-17	***Bacillus licheniformis***	**KU854963**	CTTNCNCNTCGCNANTCGTAACAAGTCGAGCGGACTGACGGGAGCTTGCTCCCTTAGGTCAGCGG CGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGG CTAATACCGGATGCTTGATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTAGCTACCACTTA CAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAG CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA GCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGG TTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGA CGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA GCGTTGTCCGGAATTATGGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTNATGTGAAAGCC CCCGGCTCAACCGGGG
BJ-DEBCR-18	***Bacillis licheniformis***	**KU854964**	CCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACT CAAAAGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGCAAAGCGAA AAACCTTACCGGGTCTTGACATCTTCTGACAACCCTAGAGATAGGGCTTCCCCTTCGGGGGCAGAG TGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC GCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCG GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAA TGGGCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGTTCTCAGTTCG GATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGC GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCGGAA GTCGTAGAGGTAATCAGTNGACCAAGCTAGCGCGTAGAGTTGA
BJ-DEBCR-20	***Bacillus pumilis***	**KX258616**	GCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCG GACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTA ATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAG ATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCG ACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTT CGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCTTGACGGT ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT TGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCG

			GCTCAACCCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTC CACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTC TGTAACTGACGCT
BJ-DEBCR-28	***Bacillus licheniformis***	**MF487826**	GCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTG GGGAACTTGAGTGCAAAAAAGGAAAGTGAATTTCCACGTGTAGCGGTGAAATGCGTAAAGATGT GGAGGAACACCAGTGGCAAAGGCGACTCTCTGGTCTGTAACTGACGCTAAGGCGCAAAAGCGTG GGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGG GGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAG ACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGC AACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCCCCTTCGG GGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCACCTCGTGTCGTGAGATGTTGGGTTAAGTCCC GCAACGAGCGCAACCCTTGATTTTAGTTGCCAGCATTCAGTTGGGCACTTTAAGGTGACTGCCGGT GACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC GTGCTACAATGGGCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGT TTTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCA GCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTA ACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCCAGCCGCCGAAGGT
BJ-DEBCR-40	***Bacillus subtilis***	**MF487838**	AGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACC TGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCAT GGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTT GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA AAGTCTGACGGAACAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGG GAAGAACAAGTACCGTTCAAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAA CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAGG GCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAA ACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAG ATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGG
BJ-DEBCR-41	***Bacillus licheniformis***	**MF487839**	GCGNTGCCTATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTCAGCGGCGGAC GGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATA CCGGATGCTTGATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTAGCTACCACTTACAGATG GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACC TGAGAGGGTGATCGGCCACACTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCAGCAGTA GGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGG ATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTAC CTAACCAGAAAGCCACGGCTAACTACGTGCCACCACCCGCGGTAATACGTAGGTGGCAAGCGTTG TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGC

			TCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAAAAAAGGAGAGTGGAATTCCAC GTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGT AACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCC GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGC ACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCC

Fermented Product: *Hungrii*

Isolate Name	Closest related microorganism	Genbank Accession No.	Sequences
BJ-DEBCR-11	***Bacillus pumilis***	**KU301334**	TCGGTCTGGGTCAGTAGGTGATGTTAAGACATAGCATTACGAGCGGACAGAAGGGAGCTTGCTCC CGGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCC GGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTC GGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCC TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTG AGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGC TTGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTG ATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAAAAGA GGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAAACACCAGTGGCGA AGGCGA
BJ-DEBCR-19	***Bacillus licheniformis***	**KX258615**	TCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTA ACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCG CATGGTTCAAATATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTA GTTGGTGAGGTAACGGCTCACCAAGGCAACAATGCGTAGCCAACCTGAGAGGGTGATCGGCCACA CTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACG AAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAG GGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTA ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAA GCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCACCCGGGGAGGGTCATTGG AAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAG AGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAA GCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCC

BJ-DEBCR-23	***Bacillus subtilis***	**MF487821**	TAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAG TAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATG GTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGC GGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGG GTGATCGGCCACACTGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAGCAGTAGGGAATCT TCCGCAATGGACGAAAGTCTGACGGAACAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAA GCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAG AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAAT TATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAAGCCCCCGGCTCAACCG GGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGC GGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGAC GCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG ATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCACTAAGCACTCCGCC TGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAG CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATTCAAG AAATAAAA
BJ-DEBCR-26	***Bacillus licheniformis***	**MF487824**	TACATGCAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAAC ACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTG ATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTACCTACCACTTACAAATGGACCCCCGGCC CATTACCTATTTGGGGAGGAACCGGCTCACCAAGGCAACAATGCTTACCCAACCTGAAAGGGTGA TCGGCCACCCTGGAACTGAAACACGGCCCAAACTCCTACGGAAGGCACCATTAGGAAATCTTCCG CATTGAACAAAAGTCTAACGAACCACCCCCCCGTGGATTGATAAAGGTTTTCGAATCGAAAAACT CTGTTGTTAGGAAAAAACAATTACCGTTCAATTAGGGGGGTACCTTGACGGTACTTAACCAAAAA GCAACGGCTAACTACTTGCCACCACCCCCGGAAATACTTAGGTGGCAAGCGTTGCCCGAAATTAT TGGCCGAAAAGCCCGCCCAGGCGGTTCCTTAATTCTAATGTGAAACCCCCCGGCTCACCCGGGAA GGGCCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTG AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG AGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
BJ-DEBCR-35	***Bacillus amyloliquefaciens***	**MF487833**	CCGAGCGGACAGATCGCGGTAGCTTGCCCCATGATGTTAGCGGCGGACGGGTGAGTAACACGTGG GTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTCTGAA CCGCATGGTTCAGACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAG CTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCC ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGG ACGAAAGTCTGACGGAGCAACGCCACGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGT TAGGGAAGAACAAGTGCCGTTCAAATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCACG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGACG TAAAGGGCTCGCAGGCNGTTTCTGAAGTCTGATGTGAAAGCCCCCGGCTCACCCGGGGAGGGTCA

			TTGGAAACTGGGGAACTTGAGTGCATAAGAGGAGAGTGAAATGCCACGAATACCGGTGAAATGC GTAGAGATGTGTAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGC GAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGTCGTAAACGATGAGTGCTAA GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAACACTCCGCCTGGGGAGTCCG GCCGCGAGGGTGA
BJ-DEBCR-36	***Bacillus licheniformis***	**MF487834**	TCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTA ACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCG CATGGTTCAAATATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTA GTTGGTGAGGTAACGGCTCACCAAGGCAACAATGCGTAGCCAACCTGAGAGGGTGATCGGCCACA CTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACG AAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAG GGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTA ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAA GCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCACCCGGGGAGGGTCATTGG AAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAG AGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAA GCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCC

Fermented Product: *Rhujuk/bastanga*

Isolate Name	Closest related microorganism	Genbank Accession No.	Sequences
BJ-DEBCR-5	***Bacillus licheniformis***	**KU301337**	AACGGAAGATGGGAGCTTGCTCCCTGATGTCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCT GCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCGCATG GTTCAATTATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTG GTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTG GGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAA AGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGG AAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGC GCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAA ACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAG ATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGC GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTA GAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGC AAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA

			AGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCCCCT TCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG TCCCGCAACGAGCGCAACCCTTG
BJ-DEBCR-9	***Bacillus subtilis***	**KU854957**	TAAGACTCGTACACTATCCGTGTCGATCGCCTTCTACAGGTATCCGTAGTCTTACGTGCCATGATG TTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAA CCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTAC CACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGAT GCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGAAACGGCCCATACTCCTACGGG AGGCAGCAGTAGGGAATCTTCCGCAATGGACAAAAGTCTGACGGAACAACGCCGCGTGAGTGAT GAAGGTTTTCGGATCGTAAATCTCTGTTGTTAGGGAAAAAAAAGTACCGTTCGAATAGGGGGTAC CTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT GGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGA AAGCCCCCGGCTCAA
BJ-DEBCR-14	***Bacillus subtilis***	**KU854961**	ATACNCNGCNNNTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGT AACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGG TTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCG GCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGG TGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTT CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAG CTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGA AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATT ATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGG GAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAAGAGAGTGGAATTCCACGTGTAGCGG TGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGC TGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCCGTAAACGA TGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCT GGGGAGTACGGTCGCAAGACTGAAACTCAAAAGGAATTGACGGGGGCCCGC
BJ-DEBCR-27	***Staphylococcus pasteuri***	**MF487825**	TTTGGGTCTTAGTATTGGTAGTGCGTCGATGCCAGTCGAGCGAAGGATAAGGAGCTTGCTCCTTTG ACGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGG AAACCGGAGCTAATACCGGATAACATATTGAACCGCATGGTTCAATAGTGAAAGGCGGCTTTGCT GTCACTTATAGATGGATCCGCGCCGTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACG ATACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACG GGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTG ATGAAGGTCTTCGGATCGTAAAACTCTGTTATCAGGGAAGAACAAATGTGTAAGTAACTGTGCAC ATCTTGACGGTACCTGATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG GTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTG AAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAA

			GTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGA CTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTG GTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTA ACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGG ACCCGCACAAGCGGTG
BJ-DEBCR-30	***Bacillus subtilis***	**MF487828**	TACATGCAAGTCGAGCGGAAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAAC ACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGAAGATGGGAGCTAATACCGGATGGTTG TTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCG CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA TCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCG TAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTC TGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAG CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT GGGCATAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAG GGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGA AATGCGTAGAG
BJ-DEBCR-31	***Bacillus subtilis***	**MF487829**	CTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTA CAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTA GCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG CAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGA AGGTTTTCGGATCGTAAAAGCTTTGTTTTTTAGGGAAGAACCAAGTCCCGTTCGCAAAGGCCGGT ACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA GGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTTTGATG TGAAACCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCCGAAGAGG AGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAG GCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATA CCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTG CAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTG ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA GGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTG GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT TGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAA GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGAC AGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATC GCAGTCTGCAACTTGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGT CGGTGAGGTAACCTTTTAGGAGCCAGCCGCCGAAGGTGGGACAGATGAT

BJ-DEBCR-32	***Bacillus amyloliquefaciens***	**MF487830**	CATGAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACG TGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTT GAACCGCATGGTTCAAACATAAAAGGTGCCTTCGGCTACAACTTAAAGATGGACCCGCGGGGCAT TAGCTAGTTGGTGAGATAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCG GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA TGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGT TGTTAGGGAAGAACAAGTGCCGTTCAAATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCA CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGG CGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGT CATTGGAAACTGGGG
BJ-DEBCR-37	***Bacillus subtilis***	**MF487835**	TTGACATGCGTGAGTGTGATGCAGTTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGG CGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGG CTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTAC AGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGC CGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAAACTCATACGGGAGGCAG CAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAACAACCCCGCGTGAGTGATGAAGGTT TTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCCAATAGGGCGGTACCTTTACG GTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGAGTGTGGCAAGC GTTGTCCGGAATTATTGGGCGTAAAAGTCTCGCAGGCGGTTTCCTAATCTTGATGTGAAAGCCCCC GGTTTAATTGGGGAGGGTCA
BJ-DEBCR-38	***Bacillus licheniformis***	**MF487836**	CNGCCAGTACGAGCGGACCGACGGGAGCTTGCTCCCTTAGGTCAGCGGCGGACGGGTGAGTAACA CGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGA TTGAACCGCATGGTTCAATTATAAAAGGGGGCTTTTAGCTACCACTTACAGATGGACCCCCGGCGC ATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGAT CGGCCACACTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGGAGCAGTAGGGAATCTTCCGC AATGGAAGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCT GTTGGTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGC CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTTGCAAGCCTTGTCCGGAATTATTG GGCGTAAAGCGCGCGGAGGCGGTTTCCTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGG GTCATTGGAAACTGGGGAACTTGAATGCAGAAGAGGAGAGTGGAATTCCACGTGTTACCGTGAAA TGCGTAGAGATGTGGAGGAACCCCCNTGGCGAAGGCGACTCTCCGTCCTGTAACTGA
BJ-DEBCR-39	***Bacillus subtilis***	**MF487837**	ACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAAC ACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTG TTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCG CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA TCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCG CAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTC

			TGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAG CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT GGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAG GGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGA AATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGA GGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG TGCTAAGTGTTAGGGGGTTTCCGCCCCCTATTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG GAGTACGGTCGCAAGACTGAAACTCAAAGGAATTTGACGGGGGCCCGCACAAGCGGGTGGAGCA TGTANNTTTTATTCGAAAGCAACGCGAAGAACCCTTACCAGGGTCTTGACATCCTCTGACAATCCC TAGAAG

Fermented Product: *Tsutuocie*

Isolate Name	Closest related microorganism	Genbank Accession No.	Sequences
BJ-DEBCR-7	***Bacillus subtilis***	**KU301335**	AGGTTGCGTGTCGACTGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGA CGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAAT ACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGAT GGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGAC CTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT AGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCG GATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTA CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT GTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGG CTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCA CGTGTAGCGGTGAAATGCGTATAGATGTGGGAGGAACACCAGTGGCGAAAGCGATTCTCTGGTCT GTAACTGACGCTGAGGAGCGAAATCGTGGGGAGCGAACACG
BJ-DEBCR-8	***Bacillus subtilis***	**KU854956**	ACNTGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAAC ACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTG TTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCG CATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA TCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCG CAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTC TGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAG CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT GGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAG

			GGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGA AATGCGTAGAGATGTGGAGGAACACCAGTGGCAAAGGCGACTCTCTGGTCTGTAACTGACGCTGA GGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG TGCTAAGTGTTAGGGGGGTTTCCCGCCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCCT GGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA
BJ-DEBCR-10	***Bacillus licheniformis***	**KU854958**	ATACCTGATTCCTGGCGGTATGCTTACAATAGCCGTCGTAACGGGGTATCCGTGTCGACGGCCTGA TGTCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGA AACCGGGGCTAATACCGGATGCTTGATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTACCT ACCACTTACAAATGGACCCGCGGCGCATTACCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACG ATGCGTAGCCGACCTGAAAGGGTGATCGGCCACACTGGGACTGAAACACGGCCCAAACTCCTACG GGAGGCAGCATTAGGGAATCTTCCGCAATGGACAAAAGTCTGACGGAGCAACGCCGCGTGAGTG ATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGG TACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA GGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATG TGAAAGCCCCCGGCTCACCGGGGAGGGTCATTGGAAACTGGGAACTTGAGTGCAGAAAGGAGAG TGAATTTCCACGTGTAGCGGTGAATGCTTAGAGTTGTGGAGGAACCCCAGTGGCGAAG
BJ-DEBCR-12	***Bacillus pumilis***	**KU854960**	TGGCGGTTGCCAGAATACCAGGAAGTCGATAAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACG GGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATAC CGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGG ACCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCT GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAG GGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGA TCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGAGTAACTGCTCGCACCTTGACGGTACCT AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTC CGGAATTATTGGGCGTAAAGGGCTCGCATGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCA ACCGGGGAGGGTCTTTGGAAACTGNGTGACTTGAGTGCAGAAGAAGACANTGGAATNCCACGTGT AGCCGTGAAATGCGTAGAGATCTCGAGGAACACCAGTGGCGAACGCGACTCTCTGGTCTGTAACT GACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCCGGTAGTCCACGCCGTAA ACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCC GCCTGGGGAGNNACNGGTCGTAA
BJ-DEBCR-25	***Bacillus subtilis***	**MF487823**	CAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCC TGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCG GGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCG GCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCG ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT ACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGA GTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGG

			CGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGTGGCAAGCGTTGTCCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCAGTTTCTTAAGTCT GATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG AGGAGAGTGGAATTC
BJ-DEBCR-34	***Bacillus pumilis***	**MF487832**	GGACTGTGTCGTATCAACTACTACCGCATGCCGCATAGATGGTGTCGTGCGGAGTATCCGGTGCTT GCTCCCGGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATA ACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACG GTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA AGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCC GCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTA ACTGCTTGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT AATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAA GTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAG AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGG CGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATT AGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGT GCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTAAC CAGGTC

APPENDIX - IV

DNA Extraction Using the CTAB protocol

Step	Action
1	5 ml of cell suspension centrifuged at 10,000 rpm for 5 min (4^oC).
2	Add 500 µL of TE buffer and mix thoroughly till pellets are completely dissolved in the buffer.
3	Add 200 µL 45 mg ml^{-1} Lysozyme and mix thoroughly. Incubate at 37^oC for 1 h.
4	Add 10 µL 20 mg ml^{-1} Proteinase K.
5	Add 50 µL 20% SDS and mix thoroughly.
6	Incubate at 37^oC until the solution becomes clear and viscous.
7	Add 100 µL 5M NaCl and incubate at 65^oC for 2 min.
8	Add 100 µL of CTAB buffer (pre-warmed at 65^o C) to each sample and incubate at 65^oC for 10 min.
9	Add an equal volume (typically 1000 µL) of Phenol:Chloroform: Isoamyl alcohol (25:24:1). Shake the tube to achieve a milky emulsion.
10	Centrifuge at 10,000 rpm for 10 min to achieve phase separation and compression of the interface.
11	Transfer aqueous phase (top layer) to a new tube without disturbing the interface.
12	Add approximately 500 µL of chilled isopropanol and centrifuge at 10,000 rpm for 15 min.
13	Decant the supernatant carefully so that the DNA pellet is not disturbed.
14	Add 1 ml of 70% ethanol to each DNA pellet and mix by carefully inverting the tube.
15	Centrifuge samples at 10,000 g for 15 min at 4^oC and discard the supernatant.
16	Re-suspend DNA pellet in 50-200 µL TE buffer.

Appendix - V

16S rRNA Universal Primer

Primer-I	9F (5'-CGCGGGATCCGAGTT TGATCCTGGCTC-3') 1492R (5'-GGCCGTCGACACGGA TACCTTGTTACGACTT-3')	Lechner et al., 1998
Primer-II	27F (5'-AGAGTTTGATCCTGGCTCAG-3') 1492R (5'-GGCTACCTTGTTACGACTT-3')	Lane, 1991

Appendix - VI

PCR Master Mix

For 25 µL Reaction		
10X Buffer with $MgCl_2$ (25 mM)	2.5 µL	1X
dNTPs (10mM)	0.5 µL	0.2mM
Forward primer (10pM)	0.5 µL	0.2pM
Reverse primer (10pM)	0.5 µL	0.2pM
DNA template (>1000mg)	5.0 µL	50-100ng
Taq DNA Polymerase (5Units)	0.2 µL	1Unit
Pure water	15.8 µL	

****