

Review Article

Probiotic microorganisms in fermented food of North Eastern States of India

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ABSTRACT

Probiotics are live microorganisms that provide health benefits when consumed in specific quantities, by improving or restoring gut flora. Probiotics balances gut microflora of host and are linked to a wide range of health benefits. Northeast region of India region has a diverse population. Ethnic people of this region use their own methods of fermenting food for the purpose of preservation. They have been practicing these methods from long time. All the fermented products are region specific and have their own unique preparation methods. Some of the most common microbes isolated from fermented foods of this region are *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Salivarius ss*, *Thermophilus*, *Saccharomyces cerevisiae*, *Lactobacilli*, *Leuconostoc mesenteroids*, *Lactobacillus brevis*, *Pediococcus pentosaceus*, *Staphylococcus sp.*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus* etc. This review work aims at summarizing available information on microorganism found in fermented food of North eastern states.

Key words: Probiotics, Fermented food, Ethnic tribes of NE states, Lactic acid bacteria,

INTRODUCTION

It is a well-known fact that fermented foods are enhanced with beneficial probiotic microbes. Probiotics are defined as “a living microbial food supplement which benefits the host by balancing the intestinal microbes” (Metchnikoff, 1907). He reported that ingested bacteria in the form of fermented food could beneficially affect the normal gut flora. One of the ancient methods of food processing and preservation is fermentation. Ethnic fermented foods are produced by the ethnic people from locally available raw materials of plant or animal sources either naturally or by adding starter

culture containing functional microorganisms which modify the substrates. Ethnic people all over the world have their own methods of fermenting food materials and they have been carrying this from time immemorial. North eastern part of India is characterized by a diverse population of people with different ethnic backgrounds. Most of the people of this region have their own methods of fermenting food materials and they have been carrying this from time immemorial. Fermentation of food is the most economical method for the development of aromas, textures and flavors as well as for preservation and biological enrichment by manipulation of different microbial populations. Wild

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fruits and vegetables have more nutritional value than that of the cultivated ones and hence, contribute to the sustainable food production and security. Fermented foods with live cultures of beneficial microbes are considered as foods with probiotic benefits. These microorganisms in fermented foods are known to balance the friendly bacteria in the digestive system. Probiotics can confer different health benefits. Common microorganisms associated with some fermented foods are listed in Table 1.

In some parts of the world fermented foods make a significant contribution to the diet of millions of individuals. This ancient food preservation process increases the shelf life of ingredients and decreases the need for refrigeration or other forms of food preservation technology. It enhances digestibility by breaking down biomolecules and enables the production of organic acids, nutritional enrichment and reduction of toxins (Sekar and Kandavel). Each fermented food in association with its distinct micro flora increases the

level of proteins, vitamins, amino acid, fatty acid and other nutrient component (Das *et al*). Microorganisms can transform the raw materials into biochemically useful products and destroy or detoxify the harmful products like phytales, tannins, and polyphenols.

Commercially, cultured dairy product manufacturers have long promoted the presence of live cultures. For many years, cultured dairy products were the only fermented foods that included label declarations regarding the presence of live microorganisms. Probiotic microorganisms from dairy products mostly belong to Lactic Acid bacteria (LAB) group. In some countries the number of live microbes presents in yogurt and other cultured dairy products must satisfy regulatory requirements. According to the CODEX standards for fermented milk products, the minimum number of starter culture bacteria in yogurt is 10^7 cfu per g (CODEX STAN 243-2003). If presence any other organisms is indicated, it must be present at 10^6 cfu per g. Recently, intake of fermented foods contain-

Table1. Some common microbes present in fermented foods.

Food	Organisms
Yogurt	<i>St. thermophilus</i> , <i>L. delbrueckii ssp. Blgaricuss</i> .
Chesse, sour cream	<i>Lc. lactis</i> , <i>Lu. mesenteroids</i>
Sausage	<i>L. sake</i> , <i>L.planatarum</i> , <i>S.carnosus</i> , <i>S.xylosus</i> , <i>P.acidlactici</i>
Wine	<i>Sa.cerevisae</i> , <i>O oeni</i>
Beer	<i>Sa.cerevisae</i> , (<i>L.brevis</i>)
Bread	<i>Sa.cerevisae</i> ,
Sourdough bread	<i>L. sanfranciscensis</i> , <i>C. humilis</i>
Saurkraut or Kimchi	<i>Lu. Mesenteroids</i> , <i>L.planatarum</i> , <i>L.brevis</i>
Olives	<i>L.planatarum</i> ,
Soy sauce, miso	<i>A. soyae</i> , <i>Z.rouxii</i> , <i>T.halophilus</i>
Tempeh	<i>R.oligosporus</i>
Natto	<i>B.subtilis var. natto</i>

(Data adopted from work of Marco *et al*).

ing live microorganisms has emerged as an important dietary strategy for improving human health (Marco *et al.*, 2017). In general, lactic acid bacteria (LAB) are predominant in fermented foods, but other bacteria as well as yeast and fungi also contribute to food fermentations. Commercially-produced fermented foods also frequently serve as carriers for probiotic bacteria. Common fermented dairy products are accepted by consumers as good sources of live bacteria. Fermented food products may also serve as a source of income to many rural people, who prepare them at home and market them locally. Detailed studies on nutritive and medicinal value of these products can provide valuable information and would prove beneficial in guiding the use of these products on a wider scale. Conservation of indigenous knowledge and proper documentation of wild edible bio-resources are suggested for sustaining the livelihood of ethnic communities. Steinkraus and Tamang suggested that globally fermented foods can be categorized into nine major groups on the basis of various substrates viz cereals, vegetables, bamboo shoots, legumes, roots/tubers, milk products, meat products, fish products, fermented products, and alcoholic beverages.

Selection of potential probiotic candidate from fermented food

A microbial strain has to undergo all the standard biochemical tests for probiotic selection. It should also be able to withstand the food processing procedure and stay alive to confer the health benefit. Only a viable microbe inside the host gut will be able to interact with gut micro flora, survive in the hostile environment and adhere to the lining of stomach and intestine. The microbial strains from fermented foods are subjected to various biochemical tests for its characterization as per ICMR-DBT Guidelines, before it can be termed as probiotic. Probiotics must meet important qualifications, including being non-pathogenic acid and bile-tolerant strains that possess the ability to act against pathogens in the gastrointestinal tract. The final stages of selection involve the accurate identification of the probiotic species. Many genera/species of microorganisms have been reported in relation to various fermented foods and beverages across the world. Basic microbiological identification procedures are used for identification of strains from all the samples after their purification by standard microbiological methods. Initial

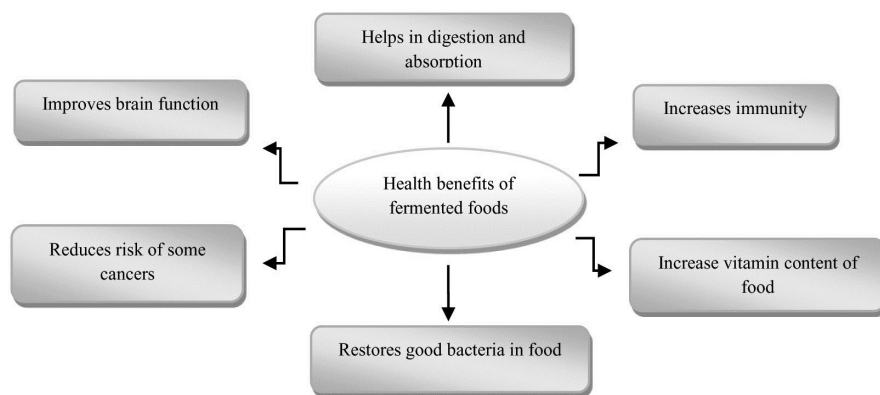


Figure 1. Health benefits of fermented food

screening of microbial strains for in vitro phenotypic tests will be performed by established protocol. Purified strains are checked for their properties to be a probiotic viz production of antimicrobial compounds, resistance to low pH and acids, hemolytic activity, bile salt hydrolysis (BSH activity), auto aggregation, bacterial surface hydrophobicity, high cell viability and survival in a simulated human digestion process. Its ability to survive through the entire GIT will be evaluated as the beneficial effects of probiotic are mainly centralized in the GI tract.

Probiotic Strains of Microorganisms in Different Fermented Food

There are many different types of fermented foods that are consumed by the people around the globe. They are Kefir, Sauerkraut, Tempeh, Natto, Cheese, Kombucha, Miso, Kimchi, Salami, Yogurt, Sourdough bread, beer, wine, olives, etc. Some common microbes found in them are listed below *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Salivarius ss*, *Thermophilus*, *Saccharomyces cerevisiae*, *Lactobacilli*, *Micrococaceae*, *Leuconostoc mesenteroids*, *Lactobacillus brevis*, *Pediococcus pentosaceus*, *Staphylococcus*, *Clostridium*, *Lactobacillus delbrueckii*, *lactobacillus acidophilus* etc. Several studies have shown the existence of several fungi such as *Saccharomyces sp.*, in different Indian fermented dairy and dairy-related products like curd, cheese, idli, dosa, jalebi, wari, pappad, Kanji, fruit juices, bakery products and brewery products (Mahasneh A.M, Abdas M.M. 2010). *Saccharomyces cerevisiae* has a QPS (Qualified Presumption of Safety) status according to EFSA (European Food Safety Authority). *Saccharomyces cerevisiae* is the only commercialized yeast which is practically used as probiotic in human medicine (Czerucka D *et al.*, 2007). Aerobic bacterial count in Miso, were reported to be 10^2 to 10^7 cfu/g while LAB counts for other fish based

fermented food were between 10^3 to 10^7 cfu/g (Rezac *et al.*, 2018). Some major groups of fermented food prepared by different communities of North eastern India and prevalent microbes in them are discussed in this review paper.

Vegetable based fermented food

Bamboo shoots have been used as a traditional fermented foods in the parts of Asian countries such as China, Japan, US, India, Thailand, Nepal, Bhutan, Korea, Malaysia and Indonesia. Bamboo shoots have been used as a part of tribal diet since ages. Bamboo shoots are rich in mineral content, low in fat and high in dietary fibres. Thakur *et al.* (2015) reported that these species of *Lactobacillus plantarum*, *L. brevis*, *L. Corniformis*, *L. fermentum*, *Leuconostoc fallax*, *Lactococcus lactis*, *L. mesenteroides*, *Enterococcus durans*, *Streptococcus lactis*, *L. Casei* and *Tetragenococcus halophilus* were primarily present in bamboo shoots based fermented foods. LAB species present in fermented bamboo shoots possess functional probiotic properties (Jeyaram *et al.*, 2010; Thakur *et al.*, 2015).

GUNDRUK is a fermented food from Arunachal Pradesh, prepared with leaves of mustard/radish/cauliflower. The microorganisms involved are *Pediococcus pentosaceus*, *Lactobacillus cellubiosus* and *Lactobacillus plantarum*. (Tamang *et al.*, 2005). SINKI is another one, prepared from radish root (*Raphanus sativus L.*) and the fermentation takes 30–40 days. The microorganisms involved in the production are *Lactobacillus fermentum* which initiates the fermentation in sinki production, followed by *Lactobacillus brevis* and *Lactobacillus plantarum*. (Tamang and sarkar, 1993). Khalpi is another fermented cucumber food product. *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc fallax* are involved. Nepali tribe prepare a sticky fermented soybean food from soybean seeds known as Kinema and *Bacillus subtilis* is a functional microorganism in Kinema fer-

mentation. *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis*, are mostly involved in fermentation of Hawaijar, which is a sticky fermented soybean food from Assam.

Young shoots of *Dendrocalamus hamiltonii*, *Dendrocalamus giganteus*, *Bambusa tulda*, *Bambusa balcooa* and *Bambusa pallida* are used to make SOIBUM. The microorganisms involved in this fermentation process is *Lactobacillus plantarum*, *L. brevis*, *L. corniformis*, *L. delbrueckii*, *Leuconostoc fallax*, *L. lactis*, *L. mesenteroides*, *Enterococcus durans*, *Streptococcus lactis*, *Bacillus subtilis*, *B.licheniformis*, *B.coagulans* and yeast *Candida*, *Saccharomyces* and *Torulopsis*. Giri and Janmejy (2000) found that longer incubation time results in a better quality of the fermented bamboo shoot products. SOIDON is a vegetable based fermented food from Manipur. The microorganisms involved are *Lactobacillus brevis*, *Leuconostoc fallax*, *L. lactis*. HIRING is a vegetable based sour fermented food, prepared from bamboo shoots. It is sour and has acidic taste. The microorganisms involved in this fermented food is *Lactobacillus plantarum* and *Lactococcus lactis* (Sonar and Halami, 2014; Das and Deka, 2012; Tamang *et al.*, 2008).

Fruit based fermented food

M. Battcock *et al* in the year 2001 with his investigation in this area reported that the consumption of lactic acid fermented fruits and vegetables supports to refine the human gut system in several ways such as the fulfillment of a balanced nutrition, receiving of vitamins, minerals, and carbohydrates, and put a stop to several diseases such as diarrhea and cirrhosis of liver because of probiotic properties. Research and development of fermented fruit has broad prospects in enriching the beverage market and increasing the vegetable value due to good flavor and nutritional health value. Ethnic tribes usually prepare different types of fruit beverages from fruits. Some of them are

fruits like Naga apple (*Docynia indica*), passion fruit (*Passiflora edulis*), plum (*Prunus sp.*) and gooseberry (*Phyllanthus emblica*). The fruits/ pulps are boiled after removing the seeds. The boiled fruits or pulp is then soaked in sugar syrup for fermentation process for 1 to 2 weeks. The fermented product is usually taken as beverage. Some other fruit based fermented products are prepared from preservation of local fruit with salt or sugar and dried. Probiotic bacteria, predominantly found in fermented fruits are *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Streptococcus faecalis*, *Pediococcus pentosaceus*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus fermentum* *etc.*

Cereal based Fermented Food

Cereals provide a natural growth media for probiotics and also protect the organisms in the hostile condition of the intestine. For this reason, the cereal based fermented foods are now more popular than the other conventional dairy-based food products, particularly in Japan and Europe (Mousumi Ray *et.al*, 2016). Ethnic people of North East India produce different cereal based fermented foods viz *Selroti*, *Jalebi*, *Sour Rice*, *Hakua* *etc.* *Selroti* is a rice-based fermented product. *Selroti* is a cholesterol free and trans-fat free food item. J.P. Tamang *et. al* (2012) found *selroti* has beneficial impact on health and can be recommended diet for protecting dyslipidemia and cardio metabolic disorder. *Selroti* fermentation process are carried out by *L. mesenteroides*, *E. faecium*, *P.pentosaceu*, *S. cerevisiae*, *S.kuyveri*, *D. kluuyveri*, *Z. rouxii*, *D. hansenii*, *P.burtonii*. *Sour rice* is famous fermented food in Assam and West Bengal which is popularly known as *poita bhat* and *panta bhat*. The main raw ingredients are rice and water. The cooked rice is cooled down to room temperature and adequate water is added to it. This watery rice is allowed to ferment overnight at room temperature. Mousumi Ray *et. al*, (2016) studied microbial species associated with *poita bhat* are LAB like

Lactobacillus bulgaricus, *Lactobacillus casei*, *Streptococcus thermophilus*, *Saccharomyces* sp, *Pediococcus acidilactici*, *S. faecalis*, *Microbacterium flavum*.

Meat Based fermented food

Fermented meat products are prepared and can be preserved for years in normal room temperature. Excess meat is processed and preserved by drying or by drying cum smoking or by salting and drying with local herbs or by fermentation. In the traditional fermentation process, acid is generated which is responsible for the enhancement of the taste and other nutritional value of the meat products. The acid generators are primarily Gram-positive acidogenic lactic acid bacteria such as *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Lactococcus* and *Enterococcus*. These can metabolize several saccharides into lactic acid, alcohol, lipids and some amino acid. Different bacteria have a different level of efficiency for metabolizing saccharides present in the meat.

Fish based fermented food

Fermented fish is a traditional method of preserving fish. Popular fermented fish products of north eastern region include Shidal, Utongkupso, Hentak and Ngari were studied to record the preparation process and biochemical and microbiological characteristics of the products.

Thapa *et al.* (2004 and 2016) studied and isolated the bacterial communities *Lactococcus lactis* subsp. *cremoris*, *L. plantarum*, *L. fructosus*, *L. amylophilus*, *L. coryniformis*, *L. plantarum*,

Enterococcus faecium, *Bacillus subtilis* and *B. pumilus*, *B. cereus*, *Staphylococcus aureus* and *Enterobacteriaceae* population in Ngari, Hentak and Tungtap.

CONCLUSION

The various fermented food products are not only rich in nutritional supplements but also rich in me-

dicinal value. The bamboo shoots are found to be associated with health benefits like anti-aging, anti-cancer, prevent cardiovascular diseases, weight loss, improves digestion, decrease blood pressure, and anti-microbial activity. Fermentation methods show a strong connection of ethnic people with nature and their assessment techniques of microbial benefits without much scientific background. Beneficial food products may be formulated in future by selecting productive microbial strains, genetic improvement, study of probiotic activity which will lead to the commercialization of fermented food products. These fermented food products contain high content of probiotic strains which preserve the food for a long period of time. Multi-institutional research will lead to the standardization of the fermented food products and extend their shelf life. As, we are now entering in the post genomic age of microbiology where many microorganisms have already been sequenced for food production, this offers a new knowledge-based method which has been used to the exploitation of bacteria for food production. As development of probiotic cultures of well documented microorganisms is need of the hour, basic information about the physiology and genetics of probiotic strains from traditional fermented food of this community relevant to their intestinal roles, functional activities, and interactions with other gut micro flora. It will also help to popularize these foods among non-consumers. It is also essential to conserve traditional knowledge of ethnic people and empower them to establish small scale food businesses. Integrated research will help in formulation of new probiotic products and help millions of people facing malnutrition, maternal and infant morbidity and mortality. These foods have long been a part of the human diet, and certainly worthy of recommendation of regular consumption. It would be detrimental to human health if fermented food uses are declined. Current challenge for food scientists will be how to manage large scale production of fermented foods without

losing the unique flavours, textures, and other traits associated with the traditional products from which they are derived. Future scientific research involving microorganisms from all types of fermented food from different communities will help to integrate micro flora of traditionally fermented food of North eastern states of India and nutritional benefits to maintain health.

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