



Original Research Article

An exploration of rich microbial diversity of rare traditional functional foods of Trans Himalayan state of India with proven additional probiotic effect

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ABSTRACT

Keywords

Cuisines,
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Traditional
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Himachal
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A rich legacy of consuming varieties of fermented food in different districts of Trans Himalayan state - Himachal Pradesh, India since ages might be due to its geographical as well as climatic conditions. Being a hilly state and having a cold weather for most of the year, probably renders this pre-digested food easier for digestion besides providing high nutritional value to the hard working people of Himachal Pradesh. The traditional rare fermented food products, such as seera, bhaturu, bari, jhol, chhang etc., are quite popular indigenous foods are best explored as far their microbial diversity is concerned. These foods are not only fermented ones but also acts as functional foods and thus experience a burst of popularity as health foods not only within the state but also in the other parts of country. But these healthful recipes are losing their sheen in the rapidly growing fast food culture. Promising health benefits and effectiveness of probiotics for preventing and treating a variety of diseases have attracted attention worldwide for inclusion of these agents as functional foods. Thus, there is an urgent need to popularize and conserve our traditional food habits as well as microbial diversity to sustain the healthy food habits. In the present study special cuisines of Trans Himalayan state - Himachal Pradesh had been prepared traditionally and evaluated for their additional probiotic health effects.

Introduction

A Trans Himalayan state - Himachal Pradesh can be considered a centre of diversified food culture of hilly states of India. More than 100 types of familiar and less familiar ethnic fermented foods and alcoholic beverages are prepared and consumed by the people of Himachal Pradesh. Different types of microorganisms ranging from filamentous fungi to alcohol

producing yeasts, Lactic acid bacteria (LAB), bacilli and micrococci are associated with fermentation and production of ethnic foods and alcoholic drinks. Ethnic foods are fermented naturally, except the alcoholic beverages which are produced by using consortia of microorganisms in the form of dry, cereal based starters. Diversity within microbial species has led to the preparation

of ethnic products with different sensory characteristics. It has been observed that functional microorganisms present in ethnic fermented foods of HP are responsible in enhancing the health promotory benefits, biopreservation of perishable foods, bioenrichment of their nutritive and therapeutic values (Sharma and Gautam, 2013). These functional microbes make food assimilation easy in the body and impart health benefits by releasing more nutritional components like vitamins, minerals, antioxidants etc. Apart from these benefits, these fermented food items contains probiotic microbes which impart probiotic health effects upon consumption. The art of fermentation practiced by common-man is continuing since ages in spite of scientific and technological revolution but largely has remained confined to rural area due to:

- i. High cost or inaccessibility of the industry-made products in remote areas.
- ii. Taste of the people for the traditional fermented products.
- iii. Their socio-cultural linkage with such products.

The know-how of the traditional processes and technologies involved in the production of fermented food products has been kept on transferred from one generation to another (Thakur et al., 2004). The main of this study is to study the different popular food items of H.P., their microbial biodiversity and impact on health of consumers.

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Materials and Methods

The study was carried out in different

districts of Trans Himalayan state - Himachal Pradesh. A number of selected food materials available were identified and their traditional methods of preparation were documented. The predominant microfloras were isolated and characterized on the basis of microscopic, physiological and biochemical characteristics using Bergey's manual. Genotypic identification was done only of selected isolates. Preparation procedures of traditional food items found in Himachal Pradesh are described below:

Fermented food products:

1. *Bhatabaru*:

Bhatabaru are prepared from wheat flour, made into viscous dough by adding water, milk, sugar and Malera (inoculum). This sweetened dough is given small oval shapes to be deep fried in oil until brown. These are prepared during religious and social ceremonies by both urban and rural population.

2. *Bhaturu*

Bhaturu is an 'indigenous bread' or 'roti' and constitutes staple diet of the Himachalis living in rural areas of H.P. The starting material is wheat flour or sometimes barley flour and inoculum used is called 'Malera'. Normal fermentation time is 2-3 hours in summers and 6-7 hours in winter. Wheat flour is kneaded with inoculum and warm water. After fermentation, rise in dough is observed. After fermentation roties are made and baked after about half an hour.

3. *Kachauri*

This is the modified form of Bhaturu as these are stuffed with spicy mixed paste

of urad dal (*Vigna mungo*). These are simply baked or deep fried. These are taken as snacks with tea. Some modifications are made in kachauri such as along with dal, opium seeds (*Papaver somniferum*); dry fruits, grinded soybean (*Glycine max*)/kulath (*Macrotyloma uniflorum*) pulses are also added. After adding these, kachauri is also known as 'Bedvin roti' in different parts of the state.

4. *Jhol*

This is one of the traditional dishes of Himachal Pradesh and prepared with curd/lassi/buttermilk. For its preparation, freshly chopped coriander leaves, cumin seeds and salt are mixed with the curd/lassi/buttermilk. The mixture constantly stirred and heated till the mixture changes color from white to pale yellow to bright yellow. It is served with rice and dal. It is very healthy hot curd soup and is also used as medicine during cold and in some stomach ailments.

5. *Seera*

Wheat grains (*Triticum aestivum* Linn. Poaceae) are soaked in water for 2-3 days so as to allow fermentation to occur by natural microflora. After 2-3 days the grains are ground, steeping is done to allow the starch grains and some proteins to settle down and then bran is separated. Starch and proteins are removed, sundried and is called 'Seera'. The dried material is made in to slurry by soaking in water, which is then poured in to hot ghee, sugar is added, cooked, and served as sweet dish/snack. Seera is highly nutritive energy rich fermented food with high medicinal value thereby being recommended for

many gynecological ailments especially in uterus bleeding to a postnatal mother.

6. Sepu bari

Sepu Bari is a very famous traditional food of some parts Himachal Pradesh of served during marriages. It is prepared by soaking urad (*Vigna mungo*) and chana dal (*Cicer arietinum*) for 24 hrs. After soaking these are washed and during washing urad dal skin is removed. Now the washed pulses are mixed and grounded well. This grounded mixture is made into big round balls, wrapped in big leaves (e.g. Turmeric leaves) and kept for 8-10-hours. After this, these round balls are boiled in water. When cooled, these are cut into pieces and finally fried. These fried pieces of dal are known as 'Sepu Bari'. To be served in marriages, sepu bari is cooked in curd along with spinach. Red chili powder, coriander powder, methi powder, garam masala, haldi and salt are added to curd, mixed well and finally mixed with Palak paste. After this the "Sepu Bari" is added to the mixture along with water, cooked for 20-30 minutes and serve hot with rice.

7. Dungal Bari

Dungal Bari is prepared from tender stems of colocasia (*Colocasia antiquorum* Schott.). Urad dal is soaked overnight to remove covering of the grains, grounded to make fine paste and salt and spices are added to it. The stems of colocasia are covered with dal paste one by one. After covering stems, these are put it in sun to half dry. Now, the stems are cut along with paste into small pieces carefully and again put the pieces in Sun to dry completely and stored in an air tight container. Recipe for

preparation of dungal bari includes, heating of oil in a pan and addition of coriander seeds, cumin seeds, chilies in oil and dungal bari along with some water to it. It is served with chapattis.

8. Mash Bari

Mash bari is a traditional food and serves as staple food for people of the rural area of state. It is prepared from urad dal (*Phaseolus mungo*). It is soaked overnight to remove coverings of the grains, grounded to make fine paste and salt and spices are added to it. After this, small round balls are made and are put in Sun to dry completely and stored in an air tight container. For dish preparation, oil is heated in a pan and coriander seeds, cumin seeds, chilies in oil are added and mash bari along with some water to it. It is served with chapattis.

9. Moong bari

Moong bari are prepared in the same manner as mash bari except that it is prepared from moong dal (*Phaseolus aureus*) instead of urad dal. It also serves as staple food in rural areas of the state.

10. Badana

Badana is a traditional food of the state which is mostly prepared in marriages. For its preparation, gram flour and rice flour are mixed together. After mixing, water is added to the flour to prepare a semi thick batter. This thick batter is kept aside for about half an hour. After this, oil is added to the batter so that it should not stick but evenly coat the back of a spoon when dipped in it. After adding oil to the batter, ghee is heated, a

perforated spoon is held a little above the hot ghee, and some batter is poured on the spoon. Tapped very lightly at edge of spoon to allow droplets to fall in the ghee. Remaining batter is poured back and spoon is wiped. Now the badana will be stirred in the ghee gently and will be fried till crisp but not brown. After that drained and put into the sugar syrup. Before draining from the syrup, badana will be kept for 3-4 minutes. In marriages, it is prepared by adding it into sugar syrup and is called as 'Badane ka Meetha'.

11. Chur ka saag

Chur ka saag is an indigenous food of the state. Rhizome is its main part which is used for saag preparation along with the leaves. Dried rhizome and leaves are soaked in hot water for some time. After the rhizome become soft, it is steam cooked for 15-20 minutes. After that, it is cooked by adding spices and is served with chapattis.

12. Patrodu

Patrodu are prepared from healthy leaves of colocasia (*Colocasia esculenta* Schott.). Dough of Bengal gram flour is mixed with grounded leaves of bhavri (*Ocimum basilus* Linn.), garlic, green chilies and salt. A leaf of colocasia is taken keeping its lower side upward and applies the dough over it properly. The leaf is covered with another leaf in reverse manner and again put paste over it and repeats this process for 4-6 leaves. Fold sides of the leaves inside and then roll them. Apply paste on all sides of bed while folding. For making it one piece tie it with thread. Rolls of leaves after applying paste are steam cooked. It will take about 20-25 minutes to cook.

Take out the leaves, let it cool and cut it into small pieces. Before serving, these pieces can be shallow fried or can be deep fried depending upon the requirement, occasion and availability of time. These are specifically prepared in rainy season in the state (Kapoor et al., 2010).

13. Tilbhoga

Tilbhoga is a type of snack served during festivals. It is prepared by heating khoa till it become soft and after it Til (*Sesamum indicum*) and dry fruits are added to it. After mixing it well, it is converted into round balls known as Tilbhoga and is served as snack during festivals and ceremonies.

14. Saryare ke Ladoo

These are the traditional food of Himachal Pradesh which are served as energy rich snacks during winters. For their preparation, flakes of bathu (*Chenopodium album*) are made, keep aside & sieved. These bathu flakes are added to jaggery along with some chopped dry fruits. After that, these are prepared into small round balls and served as snack during winters and are also prepared during some local festivals.

15. Roru

Roru is a type of snack generally served during winters and is also prepared during special occasions. It is prepared by heating khoa till it become soft and then grated coconut is added to it. After cooling, it is made into small round shaped balls and is served as snack.

16. Sundh/Panjiri

Sundh/Panjiri is energy intensive food with high medicinal value and is being given to post natal mothers. It is prepared by cooking wheat flour in ghee and is stirred till it become brown. After this, sugar, dry fruits and Kamarkas (*Salvia plebeian*) are added and is further cooked.

Biochemical analysis

All the food samples were collected from various parts of the state. Nutritional value of food samples was determined for energy, proteins, reducing sugars, total carbohydrates, fats, vitamins, amino acids and minerals (ash content) by using different standard analytical methods. Energy value was determined by A.O.A.C. (1984), Total proteins were estimated using Lowry's method (Lowry et al., 1951). Reducing sugars estimation was done by DNSA method (Miller, 1959) while the total carbohydrates were determined using Phenol-Sulphuric method (Dubois et al., 1956). Fat content and Vitamins were evaluated according to A.O.A.C. (1984) and total free amino acids of fermented and non fermented food items were examined according to Moore and Stein (1948).

Microbiological analysis

Microbiological profile of fermented foods was explored as well as antagonistic role of microflora present in the samples was also studied to fine out its direct impact on health of consumers. Predominant microflora was isolated from these samples by serial dilution of the samples followed by spread plate technique as per the conventional method using nutrient agar/MRS agar medium. After incubation at 37°C for 24 h, numerous colonies were developed on the surface of plates out of which the

predominant ones were selected. To get pure cultures of these isolates, the plates were carefully sub-cultured on nutrient agar plates and incubated at 37°C for 24 h. The colonies so developed were subjected to Gram staining (Gram, 1884) and Catalase reaction.

Antagonistic activity

Different bacterial isolates were screened for their bacteriocin activity by well diffusion method (Barefoot and Klaenhammer, 1983) against lawns of test pathogens. Bacteriocin production is measured in terms of inhibition zone size. The test pathogens selected for bacteriocin screening are *Listeria monocytogenes* MTCC 839, *Leuconostoc mesenteroides* MTCC 107, *Enterococcus faecalis* MTCC 2729, *Staphylococcus aureus* IGMC, *Clostridium perfringens* MTCC 1739, *Leuconostoc mesenteroides* MTCC 107, and *Bacillus cereus* CRI.

Probiotic attributes

The best screened isolates that showed antagonism against various food borne pathogens were checked for their probiotic potential. Different probiotic attributes like bile salt tolerance (Gilliland et al., 1984), Acidity tolerance (Walker, 2000), autoaggregation (Kos et al., 2003), hydrophobicity (Mishra and Prasad, 2005), and bacteriocin production (Barefoot and Klaenhammer, 1983) were used for determining the probiotic potential of the screened isolates. The probiotic potential of the isolates were calculated using standard score card. The probiotic potential for screened LAB isolates was calculated by following formula:

$$\text{**Probiotic potential} = \frac{\text{Observed score}}{\text{Maximum score}} \times 100$$

Genotypic Identification

The best screened bacterial isolates with high probiotic potential and hyperbacteriocin producers were identified at genomic level by using 16S rRNA gene technique.

Results and Discussion

Fermented food, a part of important food eco-system, harnesses a microbial diversity and converse the functional microbiota in the environment (Tamang, 2001). Fermented foods are nutritionally beneficial and easy to preserve. The traditional recipes prepared by the local people are generally associated with festivals or with the seasons. In the present study, recipes of popular indigenous foods have been explained. Besides method of cooking, microbial profile and nutritive value of the food items are also reported. 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 (Tamang, 1998).

On the basis of phenotypic and genotypic characteristics a rich diversity of bacteria, yeast and filamentous moulds present in the food items were identified and are described in Table 1. The commonly encountered organisms in inocula of fermented foods are *Saccharomyces cerevisiae* and *Candida* sp. *Zygosaccharomyces bisporus* and *Kluveromyces thermotolerance* were also found. The predominant bacteria in the fermented products included species of *Leuconostoc* and *Lactobacillus*. Traditional fermentation of food substrates enriches it with protein, essential amino acids and vitamins, thereby enhancing nutritive value of raw material.

Nutritional evaluation

The nutritive value of food material used in the preparation of the fermented as well as non-fermented food items has been discussed:

Cereal based fermented food products

It was observed that in ethnic foods made from cereals (with or without pulses), protein content was found to increase from 5.1 ± 0.17 to $6.2 \pm 0.02\%$, Thiamine from 0.49 ± 0.02 to 1.3 ± 0.03 mg/100g, Riboflavin from 0.001 ± 0.0001 to 0.041 ± 0.002 mg/g, Nicotinic acid from 0.011 ± 0.0017 to 0.021 ± 0.002 mg/g, Cyanocobalamine 0.002 ± 0.0002 to 0.029 ± 0.003 mg/g, Methionine from 1.1 ± 0.036 to 1.4 ± 0.03 mg/g, Threonine from 1.0 ± 0.07 to 1.4 ± 0.02 mg/g, Phenylalanine from 1.5 ± 0.026 to 2.1 ± 0.3 mg/g and Lysine from 0.56 ± 0.036 to 0.72 ± 0.04 mg/g after fermentation when compared to roti (chapati). The fermentation of dough significantly enhanced the B vitamin levels especially thiamine, riboflavin and nicotinic acid due to the increase the microflora and yeasts, most of which have the ability to produce vitamins from simple precursors (Steinkraus, 1998). Soni and Arora (2000) also reported significant increase in the water soluble B vitamins including thiamine, riboflavin and cyanocobalamin in bhalla fermentation and Warri fermentation. From these values, it is concluded that that nutritive value (especially Vitamins and amino acids) in cereal based fermented food products is greatly enhanced as compared to non fermented food items e.g. chapati.

Pulse/Legume based fermented food products

Legume and cereal meet a considerable requirement of protein and carbohydrates of

local population. In case of food enriched with pulses, energy values were found to be in a range from 334-432 KCal, Proteins in between 17.1-43.2 g, carbohydrate ranges from 20.9 to 59.6 g, Fats from 1.4 to 19.5 g / 100 g of edible portion.

Minerals are found in a range between 56 to 240 mg (Ca) and 5.3 to 1075 mg (Fe). Pulse/legume based food products being highly nutritious, easily prepared and conveniently preserved are very popular and constitute the staple diet of the rural population.

Milk/buttermilk based fermented food products

In addition to cereal and legume based traditional foods, milk based foods also constitute a vital part of diet of people of Mandi district. Jhol or kheeru is the most popular dish made in most of the parts of Mandi. In fermented milk products viz. curd and yogurt, nutritional content differ considerably as compared to milk. Calories were found to be 137 kJ as compared to milk where its value was 146 kJ.

Protein content in fermented milk products increased to 14g after fermentation as compared to 7.9 g in milk, Carbohydrates increased to 19 g from 12.8 g, Riboflavin increased from 0.4 mg to 0.6 mg, Vitamin B12 from 1.1 mg to 1.5 mg, Folate from 12.2 mg to 27.4 mg, Choline from 34.9 mg to 37.2 mg, Ca from 276 mg to 488 mg, Mg from 24.4 mg to 46.5 mg, Fe from 0.1 mg to 0.2 mg, P from 222 mg to 385 mg, K from 349 mg to 625 mg, Na from 97.6 mg to 189 mg, Total fats decreased from 7.9 g to 0.4 g, Vitamin A from 249 IU to 17.2 IU, Cholesterol from 24.4 mg to 4.9 mg while Vitamin K (0.5 mg), Thiamin (0.1 mg), Niacin (0.3 mg), Vitamin B6 (0.1 mg) remained same.

Microbial profile

Different food sources in the present study were explored to explore the diversity of microbial flora in them. Different bacteria, Yeast and filamentous moulds were isolated from food items and their phenotypic as well as genotypic characterization was done. Microbial flora present in fermented foods was tentatively identified on the basis of morphological and biochemical characteristics using Bergey's manual of Determinative Bacteriology (7th Edn.). On the basis of genotypic characteristics bacteria, yeast and filamentous moulds were identified and described in Table 1.

The predominant bacteria in the fermented products included species of *Leuconostoc* and *Lactobacillus*. Other commonly present microorganisms in inocula of most of the fermented foods were *Saccharomyces cerevisiae* and *Candida* sp. *Zygosaccharomyces bisporus* and *Kluveromyces thermotolerance* were also found. Microbial count for various fermented food used in the present study was studied and has been described in Table 2.

The indigenous microorganisms present in the inocula of food tested for their antagonistic activity against selected food borne/spoilage causing bacteria viz., *Staphylococcus aureus* IGMC, *Enterococcus faecalis* MTCC 2729, *Listeria monocytogens* MTCC 839, *Clostridium perfringens* MTCC 1739, *Leuconostoc mesenteroids* MTCC 107, and *Bacillus cereus* CRI. Among the three main microorganisms i.e., yeast, bacillus and lactic acid bacteria, bacillus and lactic acid bacteria showed a potential inhibitory spectrum ranging between (10-20mm) zones of inhibition. Probiotic attributes of these bacteria were checked and lactic acid

bacteria of genera lactobacillus were found to have very high probiotic score (Table 3). The bacteria with very high probiotic potential were identified using 16S rRNA technique and they were identified as *Lactobacillus fermentum*, *L. acidophilus*, *L. plantarum*, *L. delbreuckii* subsp. *bulgaricus*, *L. crustorum*, *L. brevis*, *L. sphaericus*. Similar study to evaluate probiotic potential of lactic acid bacteria has been done by other authors Tambekar and Bhutada (2010); Obadina et al (2006). Authors compared the probiotic potential of lactic acid bacteria with commercially available probiotic preparations viz. pre-pro kid, P-

Biotics kid, Sporlac powder, Lactobacill plus, Gastroline and Standard probiotic bacterial strains viz. *L. plantarum* (G95 a), *L. rhamnosus* (G119b) and reported that isolated strains have equal potential as that of commercially available probiotic preparations and probiotic bacterial strains. Bacteriocin producers were selected on the basis of their antagonistic pattern against food borne/spoilage causing test indicators viz., *Staphylococcus aureus* IGMC, *Listeria monocytogenes* MTCC 839, and *Clostridium perfringens* MTCC 1739(Plate1).

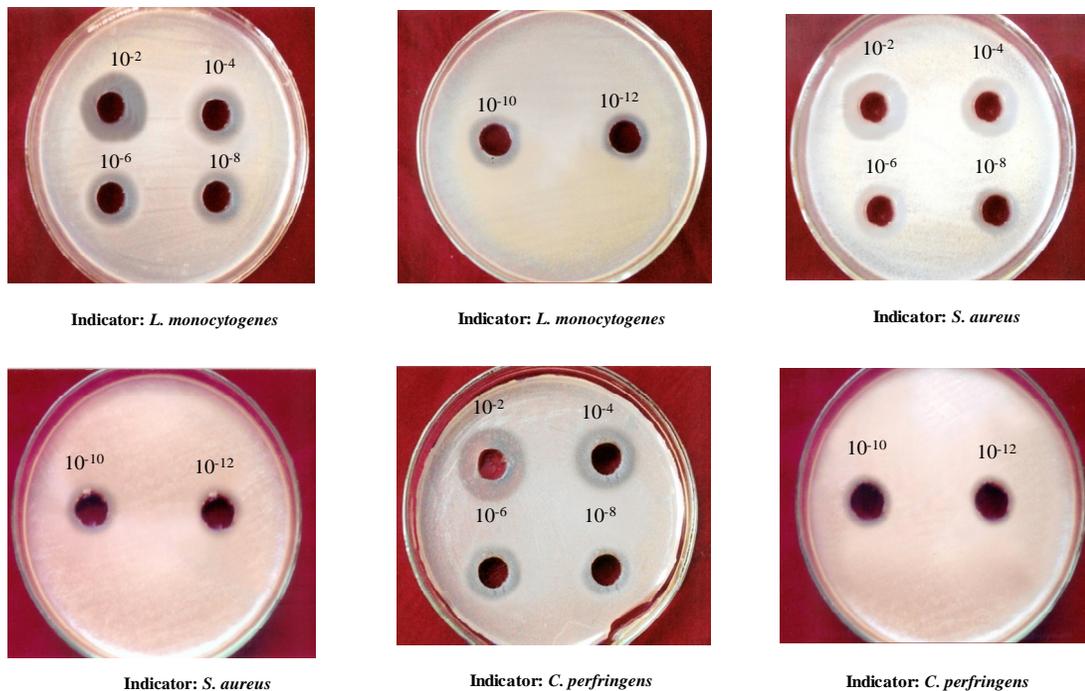
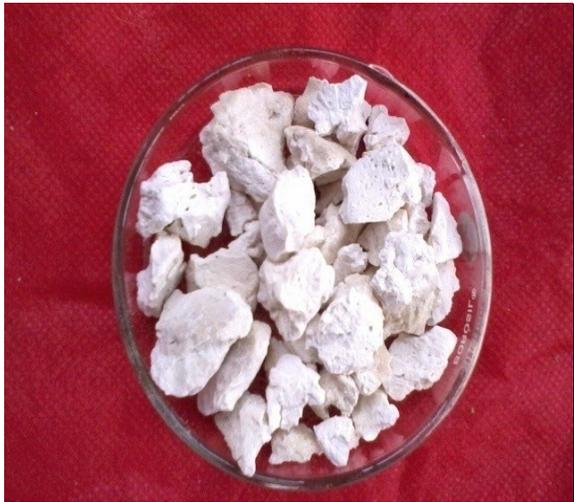


Plate 1: Detection of activity units of Purified bacteriocin- Lenticin against serious food borne pathogens



Seera



Sepu Bari



Moong Bari



Mash Bari



Kachauri

Figure.1 Fermented food products of Trans Himalayan state- Himachal Pradesh



Dungal Bari

Figure.2 Non- fermented food products of Trans Himalayan state- Himachal Pradesh



Chur Saag



Roru

Table.1 A diversity of different microorganisms present in various fermented food products

Microorganisms		
Bacteria	Yeast	Filamentous moulds
<i>Lactobacillus plantarum</i>	<i>Saccharomycopsis fibuligera</i>	<i>Mucor circinelloides</i>
<i>L. brevis</i>	<i>Saccharomycopsis crataegensis</i>	<i>Mucor hiemalis</i>
<i>L. fermentum</i>	<i>Saccharomyces cerevisiae</i>	<i>Rhizopus chinensis</i>
<i>L. bif fermentans</i>	<i>Saccharomyces bayanus</i>	<i>Rhizopus oryzae</i>
<i>L. curvatus</i>	<i>Pichia anomala</i>	<i>Rhizopus stolonifer</i>
<i>L. casei</i> subsp. <i>casei</i>	<i>Pichia burtonii</i>	
<i>L. amylophilus</i>	<i>Geotrichum candidum</i>	
<i>L. salivarius</i>	<i>Candida glabrata</i>	
<i>Leuconostoc mesenteroides</i>	<i>Candida parapsilopsis</i>	
<i>Pediococcus pentosaceus</i>	<i>Candida bombicola</i>	
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	<i>Candida chiropterorum</i>	
<i>Lactococcus plantarum</i>	<i>Candida castellii</i>	
	<i>Candida valida</i>	
Endospore-forming rods:		
<i>Bacillus subtilis</i>		
<i>Bacillus pumilus</i>		
Aerobic coccus:		
<i>Micrococcus</i> spp		

Table.2 Viable count of microorganisms found in different fermented food items

Food Product	Microorganisms	CFU/ml
Bhaturu	<i>Lactococcus plantarum</i>	60×10^6 CFU/g
	<i>Saccharomyces cerevisiae</i>	37×10^6 CFU/g
Dough	<i>Bacillus</i> spp.	47×10^6 CFU/g
	<i>Lactobacillus plantarum</i>	49×10^6 CFU/g
	<i>Saccharomyces cerevisiae</i>	22×10^4 CFU/g
Lassi	<i>Lactobacillus fermentum</i>	34×10^6 CFU/g
	<i>Lactobacillus acidophilus</i>	20×10^6 CFU/g
	<i>Bacillus</i> spp.	22×10^6 CFU/g
	<i>Saccharomyces cerevisiae</i>	17×10^6 CFU/g
Till bhoga	<i>Micrococcus</i> spp.	7.9×10^5 CFU/g

Table.3 Cumulative probiotic potential (Score Card)

Score									
Probiotic characters	Indication	<i>Lactobacillus fermentum</i>	<i>L. acidophilus</i>	<i>L. plantarum</i>	<i>L. delbreuckii</i> Subsp. <i>bulgaricus</i>	<i>L. crustorum</i>	<i>L. brevis</i>	<i>L. sphaericus</i>	<i>Lactobacillus</i> sp.
Acidity Tolerance	Resistant = 1 Sensitive = 0	1	1	1	1	1	1	1	1
Bile salt tolerance	Resistant = 1 Sensitive = 0	1	1	1	1	1	1	1	1
Autoaggregation capacity	Positive = 1 Negative = 0	1	1	1	1	1	1	1	1
Hydrophobic Capacity	(Xylene/Toluene) >40% Strong = 1 >20% Moderate = 0.5 <20% Low = 0	0.5	1	1	1	0.5	1	0.5	1
Antagonistic activity	5-10 = 0.25 10-20 = 0.50 15-20 = 0.75 >20 = 1	1	1	0.75	0.75	1	1	1	0.50
Antibiotic sensitivity	Sensitive = 1 Resistant = 0	1	1	1	1	1	1	1	1
Total		5.5	6	5.75	5.75	5.5	6	5.5	5.5
Probiotic Potential (%)		91.7	100	95.8	95.8	91.7	100	91.7	91.7

Table.4 Nomenclature of bacteriocin and their sources

Producer isolate*	Name of bacteriocin	Sources	AU/ml
<i>B. lentus</i> (Sharma et al., 2011, 2006)	Lenticin	Dough	4×10^6
<i>L. brevis</i> A ₇₅ (Gautam and Sharma, 2009)	Brevicin	Kandal vari	4×10^6
<i>Bacillus</i> sp. A 12 (Sharma et al., 2009)	Bacicin A12	Whey	4×10^6
<i>B. subtilis</i> (Sharma et al., 2011)	Subtilin R 75	Mung bari	5×10^6
<i>B. borstelensis</i> AG1 (Sharma et al., 2011)	Basicin AG1	Marcha	4×10^5
<i>B. mycoides</i> A ₂₂ (Sharma and Gautam, 2007)	Mycosin	Whey	2.6×10^6
<i>Lactobacillus brevis</i> UN (Gautam et al., 2013)	Lacticin	Dhuliiachar	8×10^3

Bacteriocin producing isolates were identified by using 16S rRNA gene technique. The nomenclature of different bacteriocins that were isolated and purified in our research laboratory has been enlisted in Table 4. These hyperbacteriocin producers were isolated from traditional fermented foods of Himachal Pradesh and they have been found to be potent bacteriocin producers with AU/ml ranging between 8×10^3 to 5×10^6 rendering them safe to be used as biopreservative in enhancing shelf stability and safety of food products. Therefore, fermented foods rich in fermenting microbes' viz. lactic acid bacteria, yeast and other bacteria can play an important role in improving human health. Microorganisms transform the raw material both biochemically (i.e., the nutrients) and organoleptically (i.e., the taste/texture/odour) into edible products that are culturally acceptable to the maker and consumers. Lactic acid bacteria (LAB) are most commonly found microorganisms in food and used for preservation of foods. Bacteriocins secreted by these bacteria are mainly responsible for preservation of food products. Fermentation by microbes in food products bring about many significant changes (Sharma, 2012). Generally, a significant increase in the soluble fraction of a food is observed during fermentation. The quantity as well

as quality of the food proteins as expressed by biological value, and often the content of water soluble vitamins is generally increased, while the antimicrobial factors show a decline during fermentation (Paredes-Lopez and Harry, 1998). Fermentation results in a lower proportion of dry matter in the food and the concentration of vitamins, minerals and protein appear to increase when measured on a dry weight basis (Adam and Moss, 1995).

Microflora in fermented foods serves as a rich source of single cell protein also as they contain high content of protein and other nutrients. After fermentation, bacteria and yeast bring about changes in nutritional content and results in increased amounts of nutrients generally proteins and vitamins. Lactic acid bacteria found in fermented foods serves as probiotics and thereby converting fermented foods to functional foods/ nutraceuticals and contributing to intestinal microbial balance. Probiotic organisms produce inhibitory substances which act against most of the spoilage and pathogenic microorganisms. Probiotic microbes help in managing lactose intolerance, improving immune system, preventing colon cancer, lowering blood pressure, reducing *Helicobacter pylori* infection and help in reduction of cholesterol and

triacylglycerol plasma concentration. Thus, these traditional foods being full of nutritional elements and rich reservoir of health promoting microbes can combat many serious ailments.

The food habits of the people changed these days but those who have their roots to their tradition, still relish the indigenous recipes prepared from seasonal plant materials. These recipes are losing their sheen in the present era of fast food culture. So there is a need to conserve our traditional food habits, recipes as well as microbial diversity associated with them. Nutritional evaluation and microbes present in different food items provide an insight of healthful properties of these unexplored ethnic foods of Trans Himalayan state of India.

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