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REVIEW ARTICLE

Probiotic bacteria in Fermented Foods: An Asian Scenario.

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ABSTRACT

Lactic acid bacteria play a very massive role nowadays in our life. Recent advanced research studies show that Probiotic plays a big role in day-to-day health improvement all over the world. They are able to produce more nutritious and tasteful food that remains fresh for a longer period and are safe for consumption and less dependent on using artificial additives. In this paper, we are going to review various research articles which are mainly based on different probiotic products that are found in different Asian countries. We will also be going to discuss different probiotic bacteria found in those probiotic food products, and their microbiological & biochemical aspects. Pathways of lactic acid fermentation and various acids produced at different step of that pathway has also been discussed and represented. During the past few decades, the research and genetical developments on LAB have been increased as Probiotic bacteria exhibits important effects on improving Food quality and Food safety. .

Keywords: Lactic Acid Bacteria, Fermentation, Probiotics, *Lactobacillus,* Kimchi, PFG.

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INTRODUCTION

Fermentation is a very ancient technology for the preservation of foods as well as to increase the nutritional value, aroma & taste of the food. In early 6000 BC, the civilization of the Fertile Crescent in the Middle East used this technology to ferment milk, vegetables, and meat [1]. This method of preserving food helps our ancestors to survive the scarcity of fresh vegetables and food during the winter season or drought periods. In many cases, the process of production of many traditional fermented foods was mistakenly produced or unknown and those processes were passed down from generation to generation as family cultures & traditions[2].

Fermentation is a process where carbohydrates are converted into alcohol organic acids which are induced by certain kinds of microorganisms, where this microbial process is desired. Different microorganisms are responsible for the production of fermented foods with Lactic Acid Bacteria(LAB), most species of *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc*, etc in many fermented foods and beverages.LAB are most commonly studied as Probiotic bacteria. Probiotics are non-pathogenic microorganisms with beneficial effect on the host. When an adequate amount of Probiotic is ingested by a host it helps improve lactose intolerance, reduce the cholesterol level, reducing the side effects of antibiotics such as diarrhea, prevention of gastrointestinal disorders, allergic reactions, and beneficial for the overall health improvement of the host.

Probiotics are desirable microorganisms that are present in the guts of a healthy human body. It has been observed that when normal flora of the gut of a host is disturbed then the person should ingest Probiotics to regain and re-establish the balance of normal microflora in the body. The important source of probiotics is a different kind of fermented foods and beverages. World Health Organisation(WHO) and Food & Agricultural Organisation(FAO) have recommended ingesting a certain amount of vegetables and fruit in the daily routine to improve health conditions. So, the consumer prefers to eat highly nutritional, fresh, and ready-to-eat foods or drinks [3]. Fermented foods and beverages are the best matches for those criteria, as it contains functional microorganisms that increase the nutritional value of the food and also boost its aroma .and taste.

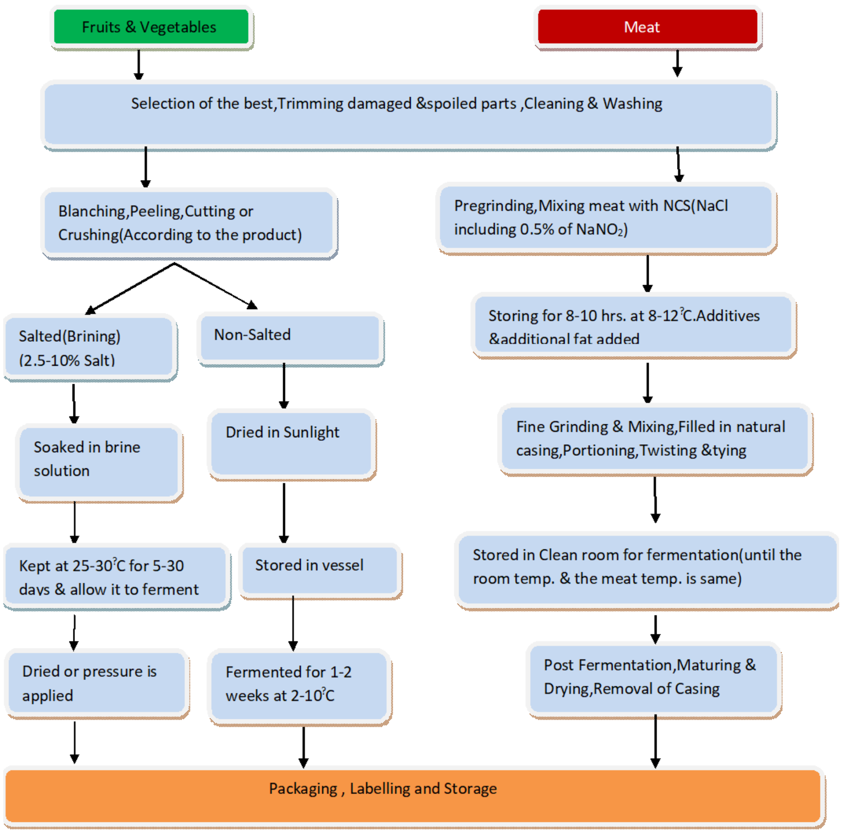


Figure 1: General fermentation process of Fruits, Vegetables and Meat[3, 19].

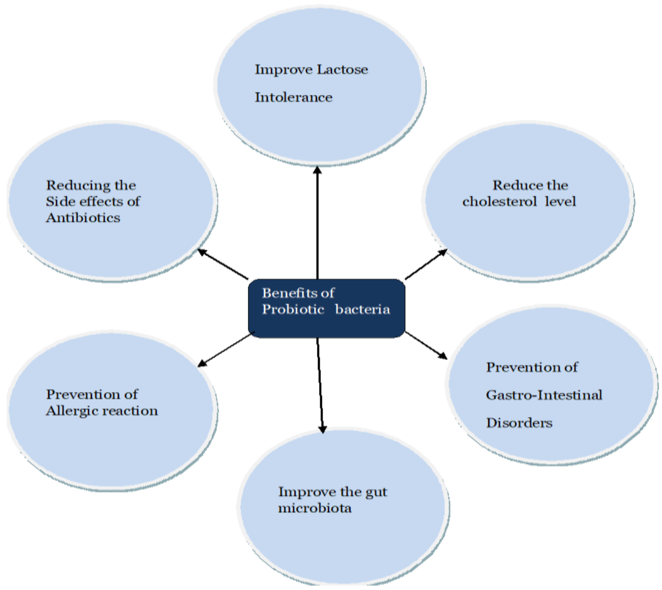


Figure2:Different Beneficiary effects of Probiotics

Microbiology Of Probiotic Bacteria

'Probiotic' is a new and interesting word added to our vocabulary that means "for life" and this word is generally preferred to name certain microorganisms that possess beneficial effects for humans. The definition states that Probiotics are live microorganisms with health benefits. The most commonly used Probiotic bacteria belong to the heterogeneous group Lactic Acid Bacteria (LAB) and the genus Bifidobacterium.

Lactic acid bacteria (LAB) represent a diverse group of bacteria such as *Lactobacillus, Lactococcus, Leuconostoc, Weisella*, etc. Even though there are some common features are present in each member of them (such as, Gram-Positive, Catalase-Negative, Non-spore former, Microaerophilic, Rods and cocci, Usually Non-motile, Produces organic acid by fermentation of raw materials mainly lactic acid) , only few specific strains of LAB are used in different fermented food products. As all of the strains are not harmless for human consumption (*Lactobacillus garvaie*caused bovine mastitis and fish lactococcosis), it is very significant to distinguish the various strains of LAB before it used in the fermented food products. *Lactobacillus lactis subsp. lactis* previously classified as *Streptococcus lactis subsp. lactis* and *Lactobacillus xylosus.* This demonstrate the fact that morphology can be deceptive in differentiation of various strains of LAB i.e. the distinction between rods and cocci is not always an easy task. *Lactobacillus cremoris* is distinguished from *Lactobacillus lactis* by certain factors such as- 1) inability to grow at 40^0C, 2) inability to grow in 4% NaCl, 3) inability to hydrolyze arginine and 4) inability to ferment ribose. It is also proved that these two strains are also genetically distinct by DNA-DNA homology studies and comparison of 16s rRNA sequences.

The Genus Lactobacillus is a heterogeneous group of LAB. Lactobacilli is currently applied as Probiotics, silage inoculants as starters in fermented food. Lactobacillus is gram-positive bacteria and rod-shaped and organized in a chain form. They developed and colonizes in anaerobic conditions and precisely fermented in nature and converts carbohydrates into lactic acid and other organic acids. Lactobacillus can interrupt the flavor and aroma of the food that is fermented. As lactic acid and other organic acids are produced by converting carbohydrates, so, the sweetness of carbohydrates will reduce and a sour taste will enhance the formation of various acids.

Lactobacillus requires a low pH of 5.5-6.5 for their growth as the main product is lactic acid. Lactobacillus has a wide range of habitats. They can find in plants, animals. raw milk or in insects. Lactobacillus is mainly used as probiotics for the preservation of foods and starters for fermented milk, vegetables, and fruits. They are also found in the gastrointestinal tract of humans as normal microflora.

They are always considered as food-grade microorganisms as they never show pathogenic properties in decades and thus they have a history of safe-use in fermented foods.

Table 1: Fermented Bacteria and their physiological Characteristics

Sl. No.	Microorganisms	pH	Temperature
1.	**Genus Lactobacillus** *Lactobacillus plantarum*	6.0	30⁰C
2.	*L. johnsonii*	5.4	37⁰C
3.	*L. acidophilus*	5.0	37⁰C
4.	*L. sakei*	5.3	30⁰C
5.	*L. bulgaricus*	5.8	35-42⁰C
6.	*L. salivarius*	6.5	37-40⁰C
7.	*L. casei*	6.4	30⁰C
8.	*L. paracasei*	5.4	30⁰C
9.	*L. rhamnosus*	4.5-6.4	6-41⁰C
10.	*L.delbrueckii*	5.4	42⁰C
11.	*L. brevis*	3.5	25⁰C
12.	*L. fermentum*	5.5-6.0	25⁰C
1.	**Genus Bifidobacterium** *Bifidobacterium infantis*	6.5-7.0	36-38⁰C
2.	*B. adolescentis*	5.5	37-41⁰C
3.	*B. animalis* subsp *animalis*	6.5-7.0	36-38⁰C
4.	*B. animalis* subsp *lactis*	6.5-7.0	37⁰C
5.	*B. bifidum*	6.5	38⁰C
6.	*B. longum*	6.5	40⁰C
1.	**Genus Saccharomyces** *Saccharomyces cerevisiae*	6.5	37⁰C
2.	*Saccharomyces bayanus*	3.0-3.5	25⁰C
3.	*Saccharomyces boulardii*	3.6	36.8⁰C
1.	**Genus Lactococcus** *Lactococcus lactis* subsp. *lactis*	3.2-4	37⁰C
1.	**Genus Streptococcus** *Streptococcus thermophilus*	6.5	43-46⁰C
1.	**Genus Enterococcus** *Enterococcus durans*	5.5	37⁰C
1.	**Genus Bacillus** *B. subtilis*	7.0-8.0	25⁰C
2.	*B. coagulans*	5.5-6.5	35-50⁰C
3.	*B. cereus*	7.0-8.0	30-40⁰C
1.	**Genus Escherichia** *Escherichia coli*	6.5-7.5	37⁰C

Characteristics of Ideal Probiotics(as cited by WHO) :

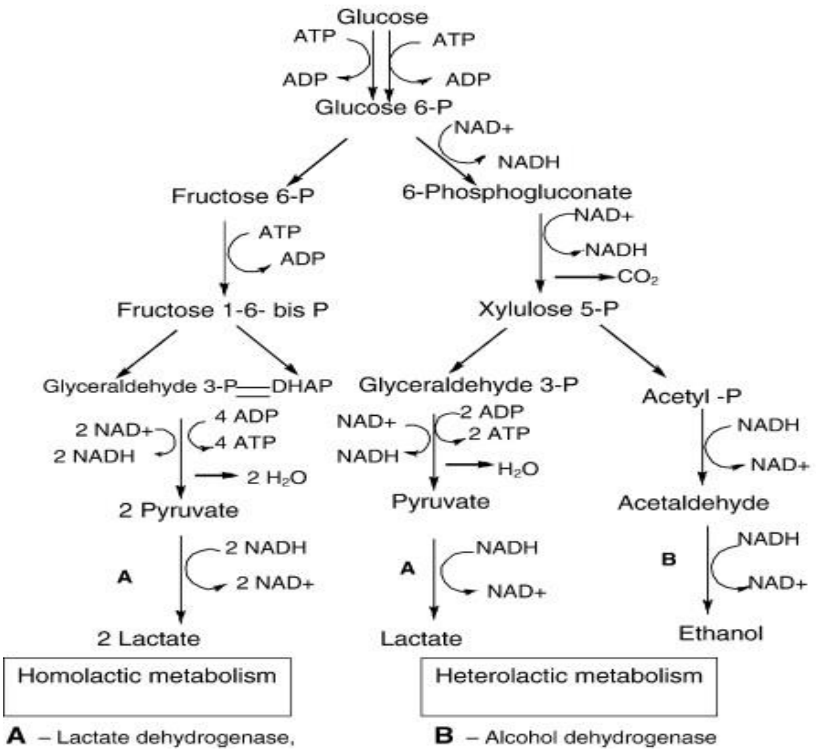
- It should be a strain, which is capable of inducing a beneficial effect on the host when ingested in an adequate amount.eg.increased growth or adequate to disease.
- It should be non-pathogenic and non-toxic.
- It should be stable and have the capability to remain viable for a longer period under storage conditions.
- It should not have the ability to transfer antibiotic resistance genes.
- It should be able to maintain genetic stability in intestinal microflora.

Biochemistry And Metabolism Of Carbohydrates In LAB

The most important characteristic of LAB metabolism is conversion of carbohydrate to lactate or lactic acid. Even though LAB degrade different carbohydrates and generally the predominant end product is Lactic acid, however when the condition differs LABnaturally and spontaneously and thus increase the shelf life &flavour of the food. This process was improved gradually and finally in the last century we get to know about the biochemistry and microbiological significance of this process. So, now we can use this process and control it more scientifically and apply on modern food technology.

Pathways of Hexose Fermentation

The primary substrate for Lactic acid fermentation is Carbohydrates (Hexoses). The two major pathways of hexose fermentation isHomofermentation and Heterofermentation. In Homofermentation, Fructose-1,6 bis phosphate is splitted into Glyceraldehyde 3-Phosphate and DHAP and then Gly-3-P convert into Pyruvate and eventually give the end product as lactic acid. Whereas in Heterofermentation, at first Glucose is phosphorylated into Glucose-6-P and then oxidation of Glucose-6-P to 6-Phosphoghluconate and then convert into Xylulose-5-P by decarboxylation,which is then splitted into Glyceraldehyde-3-P and Acetyl-P and then from Glyceraldehyde-3-P Pyruvate is formed and eventually Lactate. And usually from Acetyl-P first acetaldehyde and then Ethanol is produced.The metabolic pathway for glucose fermentation is shown in the figure below[18]:



The microorganisms which come under the genus *Lactobacillus* are generally divided into two categories depending upon their ability of carbohydrate fermentation →

1. Homofermentative →those who metabolizes carbohydrates and produce lactic acid only as a sole end product. such as *Pediococcus, Streptococcus, Lactococcus.* These microorganisms produce more than 85% of Lactic acid from glucose. 1 mole of glucose is fermented and 2 moles of lactic acid is produced, a net yield of 2 moles of ATP is generated per molecule of glucosemetabolized[2].

2. Heterofermentative →those who convert carbohydrates into lactic acid, acetic acid, other organic acids, and CO2. such as *Weisella* and *Leuconostoc*.They produce only 50% Lactic acid and ferment 1 mole of glucose to 1 mole of Lactic acid, 1 mole of ethanol, and 1 mole of CO2. One mole of ATP is generated per mole of glucose, leading to less growth per mole of glucose metabolized.

Table2: Various acids produced by Hetero-fermentation [17]

Sl.No.	Various Acids	Amount of Production(g/L)
1.	Acetate	29.94
2.	Lactate	17.11
3.	Citrate	3.40
4.	Formate	4.72
5.	Succinate	7.88

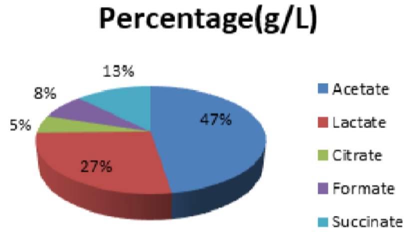


Figure 3: Pie chart showing the percentage of Acid production by heterofermentative from table 3.

Fermented Foods and Its Production

Sauerkraut: Chinese sauerkraut is one of the most famous and traditional fermented food that is prepared and consumed in China.

Preparation

Different vegetables such as cabbage, radish, tender ginger, and pepper are used for the preparation of Sauerkraut. First of all, all vegetables are cleaned and trimmed. Then, they are immersed into a 6-8% (w/v) salt solution with garlic and *Illicium verum.* Then they are kept at normal room temperature(20-25 ∘ C) for 6-10 days in airtight pickle jars. And allow it to ferment. Instead of dry salting, they are brine-salted.

Microorganisms responsible for fermentation

Lactococcus lactis, Leuconostocmesenteroides, Lactobacillus planterum, Lactobacilluscasei.[1].

Pickles: Pickles are mostly famous in Asian and African countries. It is part of the daily diet in those countries. It is produced by Lactic acid fermentation.

Preparation

Different types of fruits and vegetables are used in pickle formation. The vegetables and fruits are pressed into containers and 10-15% of NaCl solution is added to it.Vinegar is also added, and the container is kept at 20 ∘ C for fermentation for 4 weeks. Microorganisms are responsible for fermentation.

Lactobacillus planterum, Pediococcus pentosaceous[20]

The final product will have a pH of 3.4-3.6.

Kimchi: Kimchi is a very famous Korean traditional fermented vegetable, which is prepared from Chinese cabbage, radish, green onion. Kimchi is generally prepared at home as the production method is quite reasonable.it is served as a side dish with any meals. Kimchi is also produced by the process of lactic acid fermentation. But kimchi fermentation is a temperature-dependent process.

Preparation

The raw vegetables (cabbage, radish. green onion) are salted for brining. Then different kinds of spices such as ginger, red pepper, garlic powder, etc are blended with the vegetable properly. Then it is stored in a container and kept at a very low temperature 2-5 ∘ C for fermentation. Then it is kept at 15 ∘ C for one week to ripen and three days at 25 ∘ C.The temperature for kimchi fermentation is low because coldness prevents the assembly of any strong acid, overripening and it also increases its period for optimum taste.

Microorganisms are responsible for fermentation *Leuconostoc mesenteroides, Lactobacillus planterum, Lactobacillus brevis, Weisellakoreensis.*

Suan-Tsai: Suan-Tsai (Suan-cai) may be a seasonal popular dish in some parts of China and is understood for its sour taste. It can be made from cabbage or mustard. Thanks to the simplicity and flexibility of this food preparation, this will be easily produced at a really small scale in homes by spontaneous fermentation. This is often also produced on a bigger scale in some regions of China [21].

Microorganisms are responsible for fermentation →*Lactobacillus, Leuconostoc,* and *Pediococcus.*

Table 3: Country-wise list of Fermented Food Products along with Microorganisms involved.

Sl. No.	Fermented food products	Country/Region	Microorganisms involved	Reference
1.	**Vegetable-based** Sauerkraut	China, Korea	*Lactobacillus lactis, Lactobacillus planterum,*	[4]
2.	Kimchi	Korea	*Leuconostoc mesenteroides*	[2]
3.	Pickels	India,China,Japan	*Lactobacillus planterum*	[2]
4.	Peanut milk	India,China	*Streptococcus faecalis*	[5]
5.	Cabbage Juice	China,Japan	*Lactobacillus planterum, Lactobacillus casei, Lactobacillus delbrueckii*	[6]
6.	Ginger Juice	China	*Lactobacillus*	[7]
7.	Fermented Banana	Thailand	*Lactobacillus*	[8]
8.	Beets-based Drink	India, Japan	*Lactobacillus planterum, Lactobacillus rhamnosus, Lactobacillus delbrueckii*	[9]
9.	Grape and Passion fruit juice	India	*Lactobacillus acidophilus, Lactobacillus planterum, Lactobacillus helveticus*	[10]
10.	Carrot Juice	India,China	*Lactobacillus planterum, Lactobacillus pentosus, Lactobacillus bravis*	[10]
1.	**Milk-based** yogurt	India	*Lactobacillus bulgaricus & Streptococcus thermophilus*	[11]
2.	cheese	India, Japan	*Bifidobacterium longum, Bifidobacterium lactis, Lactobacillus acidophilus*	[12]
3.	Yakult	Japan,India	*Lactobacillus paracasei Shirota*	[13]
4. 5.	Ice-cream Lassi	India, China India	*Lactobacillus acidophilus* *Lactobacillus bulgaricus & Streptococcus thermophilus*	[14] [11]
6.	Curd	India	*Lactobacillus bulgaricus & Streptococcus thermophilus*	[11]
7.	Acido whey	Japan	*Lactobacillus acidophilus, Bifidobacterium animali*	[11]
8.	Cheddar Cheese	India, Japan	*Bifidobacterium longum, Bifidobacterium lactis,*	[12]
9.	**Cereal –based** Idli	Southern India	*Leuconostoc mesenteroides*	[15]
10.	Dosa	Southern India	*Streptococcus feacalis.*	[15]
11.	**Meat-based** Nem Chua	China, Vietnam	*Lactobacillus planterum, Pediococcus pentosaceus, Lactobacillus brevis*	[16]
12.	Nham	China, Japan	*Lactobacillus*	[16]

Yoghurt: Yoghurt is one quite fermented milk product produced by a special process referred to as Proto-cooperation.Proto-cooperation is a process where *Lactobacillus delbrueckii*and *Streptococcus thermophilus* are grown mutually and yogurt is produced. These interactions are known for several years. *L.bulgaricus* produces cell wall-bound proteases and successively gets to enjoy nutrients like formate and CO2 produced by *S.thermophilus*[23]. Cultivation of those organisms in milk has supported multiple events of horizontal gene transfer including the transfer of a gene cluster for the assembly of sulfur-containing amino acids from *L.bulgaricus* to *S. thermophilus*. This transfer supports combined growth and has been confirmed with the availability of multiple genome sequences[22].

Genetical Development Of LAB

The application of gene-splicing technology to enhance the existing strains or develop novel strains in the probiotic field is a lively research area throughout the world. New techniques are taking place in genetic engineerings such as the ability to sequence large chains of 16s and 23s rRNA genes using polymerase chain reaction (RAPD-PCR) and the use of pulsed-field gel electrophoresis (PFGE) to fingerprint genomic restriction patterns. This process contributed hugely to the identification and classification of strain[1]. This has a great application in the field of probiotics where the ability is to monitor the strains through clinical trials and to evaluate their effects on the gut microflora. The protection of their proprietary value depends on exact and reproducible strain identification[24].

Industrial Challenges Of Fermented Food

Due to the simultaneous present of Probiotics and acidifying starter bacterial culture used in fermentation process, it is really challenging for the industry to maintain the microbiological enumeration of probiotic bacteria in fermented food products. Even though there are various modern and advanced techniques are there to control the selective and differentiative control of probiotic bactetia such as FISH, flow cytometry and real time quantitative PCR however in most quality control labs the traditional plate count technique is used as it is very simple and easy to implement. As the Probiotic bacteria and the starter bacteria are phylogenetically very similar and they also have a similar nutritional requirement, so it is crucial to differentiate them properly before applying to any fermented food products. During the last 20 years a wide variety of culture media is experimented for this kind of differentiation and control of Probiotic bacteria.

Research Projects And Future Prospects

We all know and it is globally proved that dairy fermented foods are the best sources for ingestion of probiotics but there is a new generation waiting to obtain non-dairy fermented food products. We can have a replacement for dairy products for those who are lactose intolerant by designing new non-dairy products. In the probiotic field, Lactobacillus is mostly used probiotic. So, we can use Gene technology and relative genomics for searching and developing new strains, so that we have more than one option to choose from, with the help of gene sequencing we can improve the characteristics features, and functional properties of probiotics.

CONCLUSION

Lactic acid bacteria play a very massive role in many Asian fermented foods. Recent advanced research studies show that Probiotic plays a big role in day-to-day health improvement in different Asian countries. They can produce more nutritious and tasteful food that remains fresh for a longer period and are safe for consumption and less dependent on using artificial additives. The probiotic functions of LAB of many non-dairy food products produced by Asian countries are still under research area. We can hope in the future we will be able to produce and investigate more probiotic bacteria and their functional properties in different Asian countries.

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Conflict Of Interest

The authors declare that they have no conflict of interest in the publication.

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