Food and Nutrition Sciences, 2014, 5, 435-442
Published Online February 2014 (http://www.scirp.org/journal/fns)
http://dx.doi.org/10.4236/fns.2014.54051

The Role of Lactic Acid Bacteria in Milk Fermentation

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Received December 3rd, 2013; revised January 3rd, 2014; accepted January 10th, 2014

ABSTRACT

Species of lactic acid bacteria (LAB) represent as potential microorganisms and have been widely applied in food fermentation worldwide. Milk fermentation process has been relied on the activity of LAB, where transformation of milk to good quality of fermented milk products made possible. The presence of LAB in milk fermentation can be either as spontaneous or inoculated starter cultures. Both of them are promising cultures to be explored in fermented milk manufacture. LAB have a role in milk fermentation to produce acid which is important as preservative agents and generating flavour of the products. They also produce exopolysaccharides which are essential as texture formation. Considering the existing reports on several health-promoting properties as well as their generally recognized as safe (GRAS) status of LAB, they can be widely used in the developing of new fermented milk products.

KEYWORDS

Lactic Acid Bacteria; Milk Fermentation; Preservative; Flavour; Health

1. Introduction

Species of lactic acid bacteria (LAB) belong to numerous genus under the family of Lactobacillaceae. They represent as potential microorganisms and have been widely applied in food fermentation worldwide due to their well known status as generally recognized as safe (GRAS) microorganisms. They are also recognised for their fermentative ability and thus enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits [1-4]. Fermentation is generally considered as a safe and acceptable preservation technology of food and fermentation using LAB can be categorized into two groups based on the raw material used, non-dairy and dairy fermentation. Milk from different mammalian animals can be used in dairy fermentation to produce several products. Milk of cow followed by milk of goat and sheep are the most widely used raw materials to produce particular economic value fermented milk products worldwide. Due to the characteristics of milk that is highly perishable, the main purpose of milk fermentation using LAB is to prolong its shelf-life as well as to preserve the nutritious component of milk. It is also recognised that fermentation of milk using LAB will undoubtedly produce good quality of products with highly appreciated organoleptic attributes. Recently, there is a growing interest to develop a variety of fermented milk products for other beneficial purposes, particularly for health purposes and preventing of toxins produced by foodborne pathogens and spoilage bacteria that enter human body [1,2,5,6]. The beneficial effects of fermented milk products are produced by a variety of bioactive compounds of LAB [7].

Lactic acid bacteria represent as the most extensively studied microorganisms for milk fermentation [8-10]. The presence of LAB in milk fermentation can be either as spontaneous or inoculated starter cultures. Milk itself is known as one of the natural habitats of LAB [11,12]. Although under spontaneous fermentations the growth of LAB can not be predicted or controlled, but this procedure has been practised and carried out traditionally for years. A procedure called as backslopping is often used. There are some examples of fermented milk by LAB produced under this procedure such as those of artisanal cheese klila [13], kumis [14], iben [15] and kurut [16]. In general, the technology of milk fermentation is relatively simple and cost-effective. On the other hand, standardized fermented milk products are produced and manufactured in large-scale production under controlled conditions and become an important industrial application of LAB as starter cultures. There are some important features of LAB starters in fermented milk products. A single potential starter culture will dominate and reduce the diversity of microorganisms in fermented milk products compare to that of products under natural fermentation. In this review we focus on the potential role of LAB in milk fermentation based on their properties that support the development of fermented milk products.

2. Properties of Lactic Acid Bacteria

Milk fermentation process has been relied on the activity of LAB, which play a crucial role in converting milk as raw material to fermented milk products. In milk fermentation industry, various industrial strains of LAB are used as starter cultures. Starter cultures of LAB were obtained from a sequence activities and passed a process of isolation, selection and confirmation. Several behaviour as the characteristics of each individual selected strains of LAB has been established and used in the production of fermented milk products industrially. The most important properties of LAB are their ability to acidify milk [17] and to generate flavour and texture, by converting milk protein due to their proteolytic activities [7,18]. The mild acid taste and pleasant fresh, are characteristics of fermented milk products such as yoghurt and cheese.

2.1. Preservative Property of Lactic Acid Bacteria

Milk and fermented milk products are favorable substrates for the growth of microorganisms that may bring to spoilage condition. The most well known characteristics of LAB related to preservative property is their ability to produce acid, which in turn exhibit antimicrobial activity. Acidification of the milk protects the milk against spoilage microorganisms and proliferation of pathogens. LAB also release antimicrobial metabolites so called bacteriocins [19]. Both acids and bacteriocins are great potential to be used in food preservation, which are considered as safe natural preservatives.

2.1.1. Acid Production

Organic acids is the end product of carbohydrate metabolism produced by BAL. Homofermentative species of LAB convert sugars in milk mostly into lactic acid, whereas the heterofermentative species convert lactose into lactic acid, acetic acid, ethanol and CO_2. Production of lactic acid by LAB is strain dependend. The newly identified *Lactobacillus paracasei* subsp. *paracasei* CHB2121 was reported to produce high concentrations of L (+)-lactic acid efficiently. It produced 192 g/L lactic acid from medium containing 200 g/L of glucose, with 3.99 g/(L.h) productivity, and 0.96 g/g yield. In addition, the optical purity of the produced lactic acid was estimated to be 96.6% L(+)-lactic acid. This strain may be suitable for use in the industrial production of lactic acid [20]. A study using 10 strains of *Lactobacillus* showed that organic acid production was considerably influenced by media used. Three different media including skimmed milk, de Mann Rogosa Sharpe (MRS) broth and Jerusalem artichoke were used. The highest acidity was obtained in MRS broth and the weakest acidification was found in skimmed milk. *Lactobacillus casei* Shirota produced the highest and *Lactobacillus rhamnosus* VT1 the lowest amount of substances being estimated as titratable acidity. The study pointed at the dissimilarity of organic acid production of *Lactobacillus* strains [21]. Two *Lactococcus lactis* strains were studied for their ability to repress the growth of bacterial pathogens. The study showed that *L. lactis* subsp. *lactis* biovar. *diacetylactis* strongly inhibits the pathogenic *E. coli* and *Salmonella enteritidis* strains tested. The main inhibitory effect seemed to be associated with fast acid production which resulted in rapid pH reduction. Given these good attributes of *L. lactis* subsp. *lactis* biovar. *diacetylactis*, it can be recommended for use as a starter culture, preferably as a freeze-dried culture, to prepare cultured milk similar to naturally fermented milk [22].

In a yoghurt preservation period experiment and mould-proof accelerated testing at 4˚C, addition of 2% (v/v) *Lactobacillus casei* AST18 in yogurt completely inhibited the growth of *Penicillium* sp., which was used as indicator fungi. *L. casei* AST18 produced lactic acid and cyclo-(Leu-Pro) as antifungal compounds. The addition of *L. casei* AST18 improved the quantity of *Lactobacillus*, but the number of *Streptococcus lactis* in 2% AST18-added yoghurt decreased by 1.0 Log (cfu/mL) compared with that in the blank group. Direct use of antifungal strains as protective cultures presents important application value to the food industry [23]. LAB from raw milk of cow, goat and ewe are detected to have antifungal activity against 4 spoilage fungi, *Penicillium expansum*, *Mucor plumbeus*, *Kluyveromyces lactis* and *Pichia anomala*. Raw milk of cow and goat can be considered as reservoir of antifungal LAB. The most active colonies with antifungal activity belonged to *Lactobacillus* spp. It is suggested that their apparent specialization may be linked to organic acids and/or ethanol produced, and the tested fungi are sensitive to those molecules. Acetic acid produced by heterofermentative *Lactobacilli* certainly played a role in antifungal activity [11].

2.1.2. Bacteriocins Production

Bacteriocins are substances of protein structure, either proteins or polypeptides, that possessing antimicrobial activities and produced during the primary phase of bacterial growth. Generally bacteriocins only active against closely related bacterial species. Most of LAB bacteriocins are small (<10 kDa) cationic, heat-stable, amphiphilic and membrane permeabilizing peptides. Bacteriocins of LAB can be divided into 3 classes, 1) lantibiotics, small (<5 kDa) heat stable of peptide substances that contain the characteristic polycyclic thioether amino acids lanthionine or methyllanthionine, as well as the unsaturated amino acids dehydroalanine and 2-aminoisobutyric acid; 2) non-lantibiotics, small (<10 kDa) relatively heat stable, non-lanthionine containing membrane active peptides; and 3) bacteriocins, heat labile proteins which are in general of large molecular weight (>30 kDa) [19]. Bacteriocins can be used as partially purified or purified concentrates and supplemented to food products, although he application of bacteriocins require specific approval as food preservatives.

Among the bacteriocins produced by LAB, nisin produced by *Lactococcus lactis* spp., is the only bacteriocin that has been officially employed in the food industry and its use has been approved worldwide [19,24, 25]. Bacteriocins produced by wild *L. lactis* strains isolated from traditional starter free-cheese made from raw milk were reported as nisin A, nisin Z and lactococcin 972 [26]. *L. lactis* W8 produced nisin concomitantly when used to produce dahi, a traditional Indian fermented milk. Dahi prepared using *L. lactis* W8 showed inhibitory against *L. monocytogenes* ATCC 19111, *Salmonella typhimurium* ATCC 23565, *Enterobacter aerogenes* ATCC 13048 and *Vibrio cholerae*, however there was no inhibitory activity when cell-free supernatant of heat-treated dahi was used. *L. lactis* W8 appeared as a potent starter culture for production of fermented milk products of safe quality. Milk fermented with *L. lactis* W8 can be used as a rich source of nisin for commercial purposes [27]. Pediocin PA-1 produced by *Pediococcus acidilactici* is an equally promising biopreservative in foods as nisin. However, its indusrial scale production has not been taken up yet due to lack of a comparable scale of production. To improve pediocin production heterologous systems have been studied which have used a variety of promoters for enhanced expression, secretory proteins for fusion and peptide tags to facilitate purification. Pediocin is also an attractive antimicrobial agent against many pathogenic bacteria and hence has pharmaceutic application [28].

2.2. Flavor Formation

There are variety of fermented milk products available in the market from different parts of the world. Variation may due to the different technology applied and strains of LAB used. Cheese is among fermented milk products with high variety and may be classified based on different criteria that include the contribution of LAB strains in the ripening process. Starter cultures of LAB are responsible for the formation of cheese flavor. Several LAB are widely used and their role can be divided into starters, and non-starters, including adjunct, cultures. Main role of starter cultures is to produce acid during manufacture and also contribute to the ripening process. Non-starter cultures do not responsible to the production of acid, but they contribute more during ripening process. Flavor formation and the characteristics flavor of individual cheese varieties are develop during ripening process by both starter and non-starter LAB. All LAB are active sinergistically to produce specific flavor of the cheese products (**Table 1**). Several steps in cheese flavor development by LAB including metabolism of lactose, lactate and citrate; lypolysis that liberate free fatty acids and proteolysis where degradation of casein followed by amino acid catabolism occured [30]. Effort to improve the quality of cheese by producing cheese with specific flavor has been of increasing interest and researches focusing on the use of potential starters derived new and powerful technology have been widely carried out [18,31-33].

The most common LAB cultures used in yoghurt manufacture is *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* They, in association and synegistically, produce volatile metabolites that determine the flavour of yoghurt. The mutual benefit between them occured by releasing the amino acids from the milk as well as organic acids and therefore they produced more lactic acid and aromatic compounds [34]. Flavour of yoghurt are supported by various compounds, in which lactic acid represents as the major contributor, and other aroma compounds (**Figure 1**).

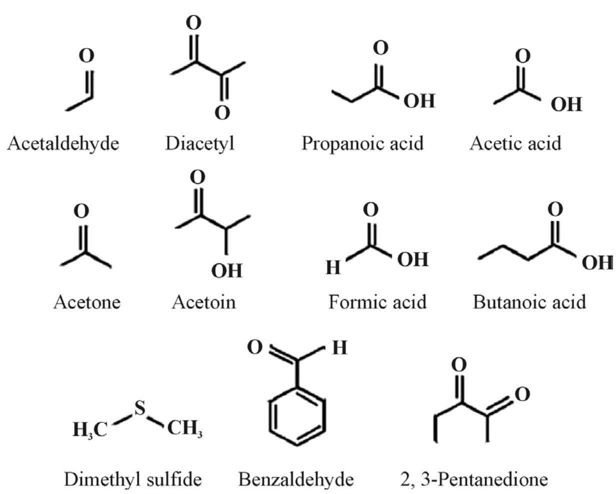

Figure 1. Major aroma compounds in yoghurt [34].

Table 1. Major aroma compounds in cheese derived from amino acids [29].

Amino acids	Aldehydes	Alcohols	Carboxylic acids	Thyol/divers
Leucine	3-Methylbutanal or Isovaleraldehyde	3-Methylbutanol	3-Methylbutanoic acid or isovaleric acid	
Isoleucine	2-Methylbutanal	2-Methylbutanol	2-Methylbutanoic acid	
Valine	2-Methylpropanal or isobutyraldehyde	2-Methylpropanol	2-Methylpropanoic acid or isobutyric acid	
Phenylalanine	Phenylacetaldehyde, benzaldehyde (-2C)	Phenylethanol	Phenylacetic acid	
Tyrosine	OH-Phenylacetaldehyde, OH-benzaldehyde (-2C)	OH-Phenylethanol	OH-Phenylacetic acid	*p*-cresol, phenol
Tryptophane	Indol-3-acetaldehyde, indol-3-aldehyde	Tryptophol	Indol-3-acetic acid	Skatole, indole
Methionine	3-Methylthiopropanal, or methional	3-Methylthiopropanol	3-Methylthiopropionic acid	Methanethiol

2.2.1. Starter Cultures of Lactic Acid Bacteria for Cheese Production

Starter cultures of LAB can be either mesophilic from the genera of *Lactococcus* and *Leuconostoc* or thermophilic from the genera of *Streptococcus* and *Lactobacillus* [35]. Among species, *Lactococcus lactis* [36,37], *Streptococcus thermophilus* [30,38] and *Lactobacillus helveticus* [36-42] are intensively studied. *L. helveticus* is specialized of milk species and belong to the member of dairy niche species [43,44] Several cheese products are based on *L. helveticus* as starter (**Table 2**). It is also known that *L. helveticus* have significant role in production of specific flavor compounds in Italian cheese types [46,47] and debittering of cheese [48,49].

Table 2. The use of *L. helveticus* as cheese starter [45].

No	Cheese product	Type of starter
1.	Asiago	Natural whey and milk culture
2.	Canestrato Pugliese	Natural whey culture
3.	Emmental	Commercial culture
4.	Grana Padano	Natural whey culture
5.	Gruyère	Commercial culture
6.	Montasio	Natural whey culture
7.	Mozzarella	Natural culture and commercial culture
8.	Parmigiano Reggiano	Natural culture
9.	Pecorino Romano	Natural culture in scotta
10.	Pecorino Sardo	Natural whey and milk culture
11.	Pecorino Siciliano	Natural whey culture
12.	Provolone Italiano	Natural whey culture
13.	Sbrinz	Commercial culture
14.	Taleggio	Commercial culture

2.2.2. Non Starter Cultures of Lactic Acid Bacteria

Non starter LAB play important role in cheese ripening. Non starter LAB released enzymes that participate in the basic role of the transformation of curd in cheese. Since the population of non starter LAB is uncontrol, it is suggested that selection of strains to be developed to maintain a certain cheese flavour [50]. In traditional cheeses, their flavor intensity also from the non-starter LAB [51].

2.3. Texture Development

Youghurt is a special fermented milk product which have a texture of soft and thicker compare to that of its raw material, the milk. The mild acid with pleasant fresh taste supported characteristics of yoghurt as an extraordinary fermented milk product. The texture of yoghurt is supported by production of exopolysaccharides (EPS), as viscosifying agent, produced by LAB. It is also suggested that is contributed by coagulation, as a result of neutralisation of the negative charges on the milk proteins, as another effect of acid produced by LAB. EPS is produced by some of LAB, depending on the strain [52].

2.4. Health-promoting Property of Lactic Acid Bacteria

The high demand of fermented milk products is due to the health property generating from consumption of fermented milk products. Bioactive peptides produced from hydrolysis of casein in milk generated by *L. helveticus* have been reviewed and showed effect of antihypertensive, immunomodulatory activity, anti-cancer and calcium binding ability. *L. helveticus* is known as one of LAB which has efficient proteolytic system [7]. Fermented milk products are reported to contribute to human health through several mechanisms [2] LAB are in the first rank of listed organisms as species used in probiotic preparation. LAB, in general, showed to possess most of the requirements for strains to be called as probiotics. [53-57]. Fermented milk products with their beneficial effect are presented in **Table 3**.

3. Concluding Remarks

LAB are widely applied to several milk products due to

Table 3. **Functional benefit of fermented milk products using lactic acid bacteria.**

Product name	Origin	Culture	Functional benefit	Reference
Probiotic yogurt	Ontario, Canada	*L. rhamnosus* CAN-1	Nutrition and immune function for people living with HIV	[58]
Mix ewe's and goat's milk yoghurt	Antakya-Hatay, Turkey	*S. thermophilus* and *L. delbrueckii subsp. bulgaricus* (codes: CH-1 and YF-333)	High short chain free fatty acids	[59]
Ayran (yoghurt from goat milk)	Turkey	*L. plantarum*, *L. brevis* *L. paracasei* subsp. *paracasei*, *L. casei* subsp. *pseudoplantarum*	High exopolysacharide	[60]
Gioddu, traditional fermented sheep or goat milk	Sardinian, Italy	*S. thermophilus*, *L. lactis subsp. lactis* *L. delbrueckii subsp. bulgaricus*, *L. casei subsp. casei*, *L. mesenteroides subsp. mesenteroides*	Probiotic	[61]
Tarag	Mongolia	*L. helveticus*, *L. lactis* subsp. *lactis*, *L.casei*	Probiotic	[62]
Fermented milk	Japan	*L. casei* strain Shirota	Maintenance treatment for myelopathy/tropical spastic paraparesis (HAM/TSP) patients	[63]
Koumiss from mare's milk	Italy	*L. delbrueckii* subsp. *bulgaricus S. thermophilus*	Antiallergic	[64]
Lben	Marocco	Spontaneously/not identified	Low fat and high calcium traditional product	[65]
Functional fermented milk	Italy	*L. lactis* DIBCA2, *L. plantarum* PU11	Enriched of Angiotensin-I Converting Enzyme (ACE)-inhibitory peptides and G-amino butyric acid (GABA)	[66]
Kumis	West Colombia	*E. faecalis*, *E. faecium*	ACE Inhibitor	[67]
Ewe milk, traditional yoghurt	Iran	*L. brevis*	cholesterol reduction	[68]
Maasai	Kenya	*L. plantarum*, *L. fermentum*, *L. acidophillus*, *L. paracasei*	Diarrhoea and constipation	[69]
Suusac	Kenya	*L. curvatus*, *L. plantarum*, *L. salivarius*, *L. raffinolactis* *Leuconostoc mesenteroides subsp. mesenteroides.*		[70]

their specific properties. Both starter and non-starter are promising cultures to be explored in fermented milk manufacture. Considering the important status as GRAS microorganisms, LAB can be used widely in the developing of new fermented milk product.

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