*Available online at* www.scholarsresearchlibrary.com

Scholars Research Library

**Scholars Research Library**

**Annals of Biological Research, 2010, 1 (4) : 218-228**
***(http://scholarsresearchlibrary.com/archive.html)***

Annals of Biological Research
Scholars Research Library
**ISSN 0976-1233**
**CODEN (USA): ABRNBW**

# Lactic acid bacteria: their antimicrobial compounds and their uses in food production

**P. Rattanachaikunsopon and P. Phumkhachorn***

*Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Warinchamrap, Ubon Ratchathani, 34190, Thailand*

---

## ABTRACT

*Lactic acid bacteria are a group of gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. They consisted of many genus including Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus, and Weissella. Lactic acid bacteria have been used in the production of foods, especially fermented foods because they can produce several compounds that contribute to taste, smell, color, and texture of the foods. In addition, they can produce antimicrobial substances including bacteriocins that have ability to inhibit pathogenic and food spoilage bacteria. This review begins with some important characteristics of lactic acid bacteria and their uses in foods. Then, it focuses on the antimicrobial substances produced by lactic acid bacteria, especially bacteriocins. Last but not least, the use of these bacteria as starter cultures in food fermentation is described.*

***Keywords:*** *Lactic acid bacteria, bacteriocin, starter culture*

---

## INTRODUCTION

Lactic acid bacteria (LAB) are a group of gram-positive bacteria including the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Streptococcus.* The general description of the bacteria included in the group is gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. Lactic acid bacteria are nutritionally fastidious, requiring carbohydrates, amino acids, peptides, nucleic acids and vitamins. Recent taxonomic revisions of these genera suggest that the lactic acid bacteria comprise the following: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella* [1]. The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or

alkaline tolerance. The lactic acid bacteria can be mainly divided into two groups based on the end-products formed during the fermentation of glucose (Figure 1). Homofermentative lactic acid bacteria such as *Pediococcus, Streptococcus, Lactococcus* and some lactobacilli produce lactic acid as the major or sole end-product of glucose fermentation. Homofermentative lactic acid bacteria use the Embden-Meyerhof-Parnas pathway to generate two moles of lactate per mole of glucose and derive approximately twice as much energy per mole of glucose as heterofermentative lactic acid bacteria. Heterofermentative lactic acid bacteria such as *Weissella* and *Leuconostoc* and some lactobacilli produce equimolar amounts of lactate, $CO_2$ and ethanol from glucose via the hexose monophosphate or pentose pathway [2, 3].

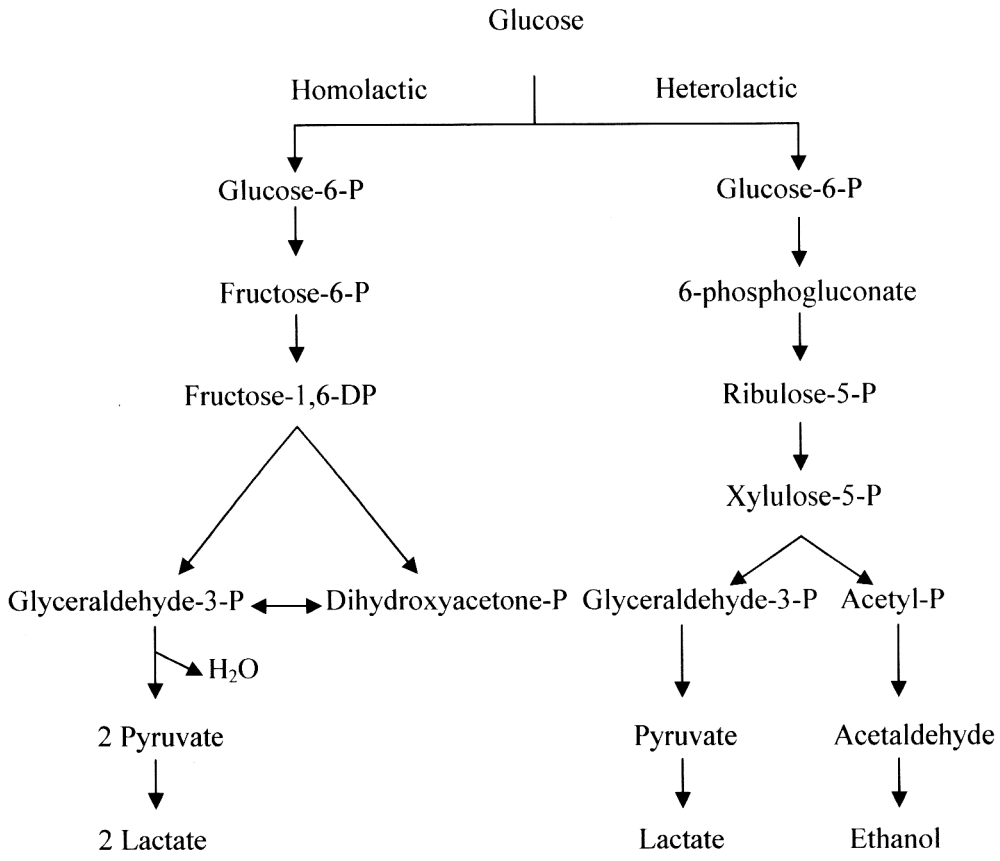


**Figure 1: Generalized scheme for the fermentation of glucose in lactic acid bacteria [6].**

## Lactic Acid Bacteria and Their Uses in Food

Lactic acid bacteria are industrially important organisms recognized for their fermentative ability as well as their health and nutritional benefits [4]. Species used for food fermentations belong to the genera *Lactococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus,* and the newly recognized *Carnobacterium* (Table 1). These organisms have been isolated from grains, green plants, dairy and meat products, fermenting vegetables, and the mucosal surfaces of animals [5]. Once used to retard spoilage and preserve foods through natural fermentations, they have found commercial applications as starter cultures in the dairy, baking, meat, vegetable, and alcoholic beverages industries. They produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic fermentations [5]. Not only are these components desirable for their effects on food taste, smell, color and texture, but they also inhibit undesirable microflora. Hence, lactic acid bacteria and their products give fermented

foods distinctive flavors, textures, and aromas while preventing spoilage, extending shelf-life, and inhibiting pathogenic organisms.

**Antimicrobial Compounds Produced by Lactic Acid Bacteria**

The preservative action of starter culture in food and beverage systems is attributed to the combined action of a range of antimicrobial metabolites produced during the fermentation process [6]. These include many organic acids such as lactic, acetic and propionic acids produced as end products which provide an acidic environment unfavourable for the growth of many pathogenic and spoilage microorganisms. Acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions [7]. They have a very broad mode of action and inhibit both gram-positive and gram-negative bacteria as well as yeast and moulds [6]. One good example is propionic acid produced by propionic acid bacteria, which has formed the basis for some biopreservative products, given its antimicrobial action against microorganisms including yeast and moulds. Microgard™ is a Food and Drug Administration (FAD)-approved fermentate produced by *Propionibacterium freudenreichii* subsp. *shermanii* which contains propionic acid and is used in an estimated 30% of the cottage cheese manufactured in the United State [8]. In addition to acids, starter strains can produce a range of other antimicrobial metabolites such as ethanol from the heterofermentative pathway, $H_2O_2$ produced during aerobic growth and diacetyl which is generated from excess pyruvate coming from citrate [8]. In particular, $H_2O_2$ can have a strong oxidizing effect on membrane lipids and cellular proteins and is produced using such enzymes as the flavo protein oxidoreductases NADH peroxidase, NADH oxidase and α-glycerophosphate oxidase [9]. Obviously, each antimicrobial compound produced during fermentation provides an additional hurdle for pathogens and spoilage bacteria to overcome before they can survive and/or proliferate in a food or beverage, from time of manufacture to time of consumption. Since any microorganism may produce a number of inhibitory substances, its antimicrobial potential is defined by the collective action of its metabolic products on undesirable bacteria.

Other examples of secondary metabolites produced by lactic acid bacteria which have antagonistic activity include the compound reuterin [10] and the recently discovered antibiotic reuterocyclin [11], both of which are produced by strains of *Lactobacillus reuteri*. Reuterin is an equilibrium mixture of monomeric, hydrated monomeric and cyclic dimeric forms of β-hydroxy-propionaldehyde. It has broad spectrum of activity and inhibits fungi, protozoa and a wide range of bacteria including both gram-positive and gram-negative bacteria. This compound is produced by stationary phase cultures during anaerobic growth on a mixture of glucose and glycerol or glyceraldehydes. Consequently, in order to use reuterin-producing *L. reuteri* for biopreservation in a food product, it would be beneficial to include glycerol with the strain. This approach was used to extend the shelf-life of herring fillets stored at 5°C and involved dipping the fish in a solution containing $1 \times 10^9$ cfu/ml of *L. reuteri* and 250 mM glycerol [5]. Results demonstrated that after 6-day of storage, there were approximately 100-fold-less gram-negative bacteria in the *L. reuteri* samples than in the untreated control.

More recently, the first antibiotic produced by a lactic acid bacteria was discovered [11]. Reuterocyclin is a negatively charged, highly hydrophobic antagonist, and structural elucidation revealed it to be a novel tetramic acid. The spectrum of inhibition of the antibiotic is confined to gram-positive bacteria including *Lactobacillus* spp., *Bacillus subtilis, Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus* and *Listeria innocua.* Interestingly, inhibition of *Escherichia coli* and *Salmonella* is observed under conditions that disrupt the outer membrane, including truncated lipopolysaccharides (LPS), low pH and high salt concentrations. Since it is

well known that nisin can kill gram-negative bacteria under conditions which disturb the outer membrane [12], it is likely that there are similarities in the mode of action of nisin and this novel antibiotic.

**Table 1: Fermented foods and beverages and their associated lactic acid bacteria**

| **Fermented products** | **Lactic acid bacteria**[a] |
|---|---|
| Dairy product | |
| - Hard cheeses without eyes | *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* |
| - Cheeses with small eyes | *L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis var. diacetylactis*, *L. lactis* subsp. *cremoris*, *Leuc. menesteroides* subsp. *cremoris* |
| - Swiss-and Italian-type cheeses | *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Lb. casei*, *Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* |
| - Butter and buttermilk | *L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis var. diacetylactis*, *L. lactis* subsp. *cremoris*, *Leuc. menesteroides* subsp. *cremoris* |
| - Yoghurt | *Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* |
| - Fermented, probiotic milk | *Lb. casei*, *Lb. acidophilus*, *Lb. rhamnosus*, *Lb. johnsonii*, *B. lactis*, *B. bifidum*, *B. breve* |
| - Kefir | *Lb. kefir*, *Lb. kefiranofacies*, *Lb. brevis* |
| Fermented meats | |
| - Fermented sausage (Europe) | *Lb. sakei*, *Lb. curvatus* |
| - Fermented sausage (USA) | *P. acidilactici*, *P. pentosaceus* |
| Fermented vegetables | |
| - Sauerkraut | *Leuc. mesenteroides*, *Lb. plantarum*, *P. acidilactici* |
| - Pickles | *Leuc. mesenteroides*, *P. cerevisiae*, *Lb. brevis*, *Lb. plantarum*, *Leuc. mesenteroides*, *Lb. pentosus*, *Lb. plantarum* |
| - Fermented olives | *P. acidilactici*, *P. pentosaceus*, *Lb. plantarum*, |
| - Fermented vegetables | *Lb. fermentum* |
| Fermented cereals | |
| - Sourdough | *Lb. sanfransiscensis*, *Lb. farciminis*, *Lb. fermentum*, *Lb. brevis*, *Lb. plantarum*, *Lb. amylovorus*, *Lb. reuteri*, *Lb. pontis*, *Lb. panis*, *Lb. alimentarius*, *W. cibaria* |
| Fermented fish products | *Lb. alimentarius*, *C. piscicola* |

[a] *B.=Bifidobacterium, C.=Carnobacterium, L.=Lactococcus, Lb.=Lactobacillus, Leuc.=Leuconostoc, O.=Oenococcus, P.=Pediococcus, S.=Streptococcus, T.=Tetragenococcus, W.=Weissella*

## Bacteriocins from Lactic Acid Bacteria

Bacteriocins are ribosomally synthesized antimicrobial compounds that are produced by many different bacterial species including many members of the lactic acid bacteria [13]. Some bacteriocins produced by lactic acid bacteria, such as nisin, inhibit not only closely related species but are also effective against food-borne pathogens and many other gram-positive spoilage microorganisms [14]. For this reason, bacteriocins have attracted considerable interest for use as natural food preservatives in recent years, which has led to the discovery of an ever increasing potential sources of these protein inhibitors.

Since bacteriocins are isolated from foods such as meat and dairy products, which normally contain lactic acid bacteria, they have unknowingly been consumed for centuries. A study of 40 wide-type strains of *Lactococcus lactic* showed that 35 produced nisin [15]. Nisin is the only bacteriocin with GRAS (Generally Regarded as Safe) status for use in specific foods and this was awarded as a result of a history of 25 years of safe use in many European countries and was further supported by the accumulated data indicating its nontoxic, nonallergenic nature. Other

bacteriocins without GRAS status will require premarket approval. Therefore, bacteriocinogenic starters, particularly if used in natural fermentations, will most likely afford the best opportunities for the application of bacteriocins in near future.

The target of bacteriocins is the cytoplasmic membrane and because of the protective barrier provided by the LPS of the outer membrane of gram-negative bacteria, they are generally only active against gram-positive cells [16, 17]. In the context of fermentation, important targets include spoilers such as species of *Clostridium* and foodborne pathogens including *Listeria monocytogenes*, *Staphylococcus* spp., *Clostridium*, *Enterococcus*, and *Bacillus* spp. The permeability of gram-negative bacteria can be increased by sublethal injury including that which can occur when using ultrahigh hydrostatic pressure (UHP) and pulsed electric field (PEF) as nonthermal methods of preservation [18]. In addition, disruption of the integrity of the outer membrane through the use of food grade chelating agents such as ethylenediamine tetraacetic acid (EDTA) and citrate which bind magnesium ions in the LPS layer can increase the effectiveness of bacteriocins against gram-negative bacteria [19]. Many bacteriocins are most active at low pH [20, 21] and there is evidence that bacteriocinogenic strains can be readily isolated from fresh and fermented foods [22]. Strains may naturally produce more than one bacteriocin and heterologous expression of bacteriocins has been demonstrated in constructed strains [23]. Protein engineering has led to the development of nisin derivatives with altered antimicrobial activities or greater solubility at pH 6 than the wild-type nisin [24]. An advantage of bacteriocins over classical antibiotics is that digestive enzymes destroy them. Bacteriocin producing strains can be used as part of, or adjuncts to starter cultures for fermented foods in order to improve safety and quality.

Bacteriocins of lactic acid bacteria, according to the classification procedure proposed by Klaenhammer [25] and modified by Nes *et al*. [26], are divided into four classes (Table 2). The majority of those produced by bacteria associated with food belong to classes I and II. Some of bacteriocins isolated from lactobacilli are listed in Table 3. Most of them belong to the class II bacteriocins [27].

**Class I**

Bacteriocins of this class contain post-translationally modified amino acids and are also termed lantibiotics. The most extensively characterized of these is nisin which has GRAS status for use as a direct human food ingredient. It is produced by strains of *Lactococcus lactis* subsp. *lactis* and has a broad inhibitory spectrum against gram-positive bacteria, including many pathogens and can prevent outgrowth of *Bacillus* and *Clostridium* spores [8]. It sensitizes spores of *Clostridium* to heat allowing a reduction in thermal processing [15].

Nisin is approved for use, to varying degrees, as a component of the preservation procedure for processed and fresh cheese, canned foods, processed vegetables and baby foods, in up to 50 countries [15, 28]. Typical levels that are used in foods range between 2.5 and 100 ppm. It is most stable in high-acid foods.

The addition of a nisin-producing strain of *L. lactis* to the starter culture used in the manufacture of nitrate-free Gouda cheese has been demonstrated to result in the prevention of the outgrowth of *Clostridium tyrobutyricum* spores and it has also been shown to inhibit the growth of *Listeria monocytogenes* in cottage and Camembert cheese [29]. Harris *et al*. demonstrated the inhibition of nisin-sensitive *Lactobacillus plantarum* and the growth to maximum densities of nisin-resistant *Leuconostoc mesenteroides* in cabbage juice using two nisin-producing strains of *L. lactis* subsp. *lactis* isolated from sauerkraut [30]. Pure nisin and mutants of *L. mesenteroides*

resistant to high concentrations of nisin were used to achieve an extension of the heterolaclic fermentation and a delay in the initiation of the homolactic fermentation of sauerkraut [31]. Choi *et al*. reduced the rate of acid production in a naturally occurring Korean kimchi fermentation using low levels of nisin [32]. In meats, nisin is not as successful a preservative, but it may allow a reduction in the levels of nitrite used in cured meat products [33].

Hugenholtz *et al*. conjugatively transferred the determinants for nisin production and immunity to two components of a starter culture for Gouda cheese manufacture [34]. Both production and immunity were transferred to the citrate-utilising component, *L. lactis* subsp. *lactis* (biovar. *diacetylactis*) and immunity only to *L. lactis* subsp. *cremoris*. Cheese made with these starters showed increased protection against the development of *Clostridium tyrobutyricum* and *Staphylococcus aureus* throughout ripening.

Lacticin 3147, produced by a lactococcal isolate from Irish Kefir grains used in the manufacture of buttermilk, is effective against a wide spectrum of gram-positive bacteria [35]. Unlike nisin, lacticin 3147 is effective at neutral pH. The genetic determinants of the lacticin are located on a conjugative plasmid and have recently been transferred to strains used in the manufacture of Cheddar cheese [36]. The resulting cheese were of normal composition except that they contained no non-starter lactic acid bacteria (NSLAB). This application of lacticin 3147 will prove very useful in studying the role of these latter bacteria in developing flavour and other characteristics in cheeses.

**Class II**

This class is divided into two sub-groups of which the Class IIa is the most common. This group is composed of the pediocin-like bacteriocin with anti-listerial activity. Pediocins are produced by *Pediococcus* spp. and while they are not very effective against spores they are more effective than nisin in some food systems such as meat. Pediococci are the main starter culture used in the manufacture of American-style fermented meats and they are also important in the fermentation of many vegetables. Pediocin PA-1/AcH is the prototype bacteriocin of this class [37] and pediocin-producing cultures are readily isolated from fermented foods [38]. Many studies report the inhibition of *L. monocytogenes* by pediocins or pediocin-producing cultures in fermented sausages [39, 40] and in Italian salami [41].

Sakacin 674 produced by *Lactobacillus sake* isolated from meat and very similar to pediocin PA-1 [42] has been shown to delay or inhibit growth of *L. monocytogenes* in vacuum-packed, sliced Bologna type sausage whether added in purified form or in the form of a bacteriocin producing culture [17].

**Other bacteriocins**

The class III and class IV bacteriocins are not well characterized. The Class III bacteriocins consist of large (>30 kDa) heat-labile proteins that are of lesser interest to food scientists. This group includes Helveticin J produced by *Lactobacillus helveticus* [43] and enterolysin produced by *Enterococcus faecalis* [44]. The class IV bacteriocins consisting of complex bacteriocins that require carbohydrate or lipid moieties for activity has also been suggested by Klaenhammer [25]; however, bacteriocins in this class have not been characterized adequately at the biochemical level.

**Table 2: Classes of bacteriocins produced by lactic acid bacteria**

| Class | Subclass | Description |
|---|---|---|
| I | | Lantibiotics-small, heat stable, containing unusual amino acid |
| II | <br>IIa<br>IIb | Small (30-100 amino acids), heat stable, non-lantibiotic<br>Pediocin-like bacteriocins, with anti-listerial effects<br>Two peptide bacteriocins |
| III | | Large (>30 kilodaltons; kDa) heat-labile proteins |
| IV | | Complex bacteriocins with glycol-and/or lipid moieties |

**Table 3: Examples of bacteriocins of *Lactobacillus* species**

| Producer Strain | Bacteriocin | Spectrum |
|---|---|---|
| *L. sake* | Lactocin S | *Lactobacillus* spp.<br>*Leuconostoc* spp.<br>*Pediococcus* spp. |
| *L. sake* | Sakacin P | *Lactobacillus* spp.<br>*Carnobacterium* spp. |
| *L. sake* | Sakadin A | *Lactobacillus* spp.<br>*Carnobacterium piscicola*<br>*Enterococcus* spp.<br>*Listeria monocytogenes* |
| *L. bavaricus* | Bavaricin A | *Lactobacillus* spp.<br>*Lactococcus* spp.<br>*Pediococcus* spp.<br>*Enterococcus* spp.<br>*Listeria monocytogenes* |
| *L. acidophilus* | Lactacin F | *Lactobacillus* spp.<br>*Enterococcus faecalis* |
| *L. curvatus* | Curvacin A | *Lactobacillus* spp.<br>*Carnobacterium* spp.<br>*Listeria monocytogenes* |
| *L. helveticus* | Helveticin J | *L. helveticus*<br>*L. bulgaricus*<br>*L. lactis* |

## Lactic Acid Bacteria as Functional Starter Cultures

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The group of lactic acid bacteria occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented foods and beverages [6]. They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. They also produce acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes. In this way, they enhance shelf-life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product.

The earliest production of fermented foods was based on spontaneous fermentation due to the development of the microflora naturally present in the raw material. The quality of the end product was dependent on the microbial load and spectrum of the raw material. Spontaneous fermentation was optimized through backslopping, i.e., inoculation of the raw material with a small quantity of a previously performed successful fermentation. Hence, backslopping results in dominance of the best adapted strains. It represents a way, be it unconsciously, of using a selected starter culture to shorten the fermentation process and to reduce the risk of fermentation failure. Backslopping is still in use, for instance in the production of sauerkraut and sourdough,

and particularly for products which the microbial ecology and the precise role of successions in microbial population are not well known. Today, the production of fermented foods and beverages through spontaneous fermentation and backslopping represents a cheap and reliable preservation method in less developed countries, whereas in Western countries the large-scale production of fermented foods has become an important branch of the food industry. Moreover, the Western consumer appreciates traditionally fermented products for their outstanding gastronomic qualities.

The direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product. Strains with the proper physiological and metabolic features were isolated from natural habitats or from successfully fermented products [45]. However, some disadvantages have to be considered. In general, the initial selection of commercial starter cultures did not occur in a rational way, but was based on rapid acidification and phage resistance. These starters are not very flexible with regard to the desired properties and functionality of the end product. Originally, industrial starter cultures were maintained by daily propagation. Later, they became available as frozen concentrates and dried or lyophilized preparations, produced on an industrial scale, some of them allowing direct vat inoculation [46]. Because the original starter cultures were mixtures of several undefined microbes, the daily propagation probably led to shifts of the ecosystem resulting in the disappearance of certain strains. Moreover, some important metabolic traits in lactic acid bacteria are plasmid-encoded and there is a risk that they are lost during propagation. It is further likely that loss of genetic material occurred due to adaptation to the food matrix. The biodiversity of commercial starters has therefore become limited. This often leads to a loss of the uniqueness of the original product and the loss of the characteristics that have made the product popular [6].

In contrast, the fermentation of traditional fermented foods is frequently caused by natural, wild-type lactic acid bacteria that originated from the raw material, the process apparatus, or the environment, and that initiate the fermentation process in the absence of an added commercial starter. Moreover, many traditional products obtain their flavour intensity from the non-starter lactic acid bacteria (NSLAB), which are not part of the normal starter flora but develop in the product, particularly during maturation, as a secondary flora [47]. Pure cultures isolated from complex ecosystems of traditionally fermented foods exhibit a diversity of metabolic activities that diverge strongly from the ones of comparable strains used as industrial bulk starters. These include differences in growth rate and competitive growth behaviour in mixed cultures, adaptation to a particular substrate or raw material, antimicrobial properties, and flavour, aroma, and quality attributes. Wild strains need to withstand the competition of other microorganisms to survive in their hostile natural environment, so that they often produce antimicrobials such as bacteriocins. In addition, they are more dependent on their own biosynthetic capacity than industrial strains and harbour more amino acid converting enzymes that they play a key role in flavour formation. Such findings underline the importance of the Protected Designation of Origin (PDO) of many of these products, which is crucial from an economical point of view since they contribute to the survival of small-scale fermentation plants in a world of ongoing globalization. A recent trend exists in the isolation of wild-type strains from traditional products to be used as starter cultures in food fermentation [48].

Nowadays, the consumer pays a lot of attention to the relation between food and health. As a consequence, the market for foods with health-promoting properties, so called functional foods, has shown a remarkable growth over the last few years. Also, the use of food additives is regarded as unnatural and unsafe. Yet, additives are needed to preserve food products from

spoilage and to improve the organoleptic properties. The demand for a reduced use of additives and processing seems contradictory with the market preference for products that are fresh, safe, tasty, low in sugar, fat, and salt, and easy to prepare. In cheese-making, for instance, the use of raw milk permits the manufacture of high-value traditional artisan varieties but brings about safety risks, e.g. the development of *Listeria monocytogenes.* On the other hand, pasteurization of the milk results in loss of flavour and gives end products that are perceived by the consumer as "boring" [49]. These market trends put the food industry under pressure to look for alternatives. In food fermentation, one of the key points for intervention seems to be on the level of the starter culture. Unfortunately, industrial starter cultures lack the necessary characteristics for product diversification, and the commercial availability of new interesting starter cultures is limited. The increased understanding of the genomics and metabolics of food microbes opens perspectives for starter improvement. Through molecular biology it is now possible to express desirable and suppress undesirable properties of starter culture [49].

Recently, the use of functional starter cultures in the food fermentation industry is being explored [50]. Functional starter cultures are starters that possess at least one inherent functional property. They can contribute to food safety and/or offer one or more organoleptic, technological, nutritional, or health advantages (Table 4).

**Table 4: Typical examples of functional starter cultures or co-cultures and their advantages for the food industry**

| **Advantage** | **Functionality** | **Lactic acid bacteria**[a] |
|---|---|---|
| Food preservation | Bacteriocin production | |
| | - Dairy products | *L. lactis* subsp. *lactis, Enterococcus* spp. |
| | - Fermented meats | *Lb. curvatus, Lb. sakei, P. acidilactici, E. faecium* |
| | - Fermented olives | *L. plantarum* |
| | - Fermented vegetables | *L. lactis* |
| Organoleptic | Production of exopolysaccharides | Several lactobacilli and streptococci |
| | Production of amylase | Several lactobacilli |
| | Aroma generation | Several strains |
| | Enhanced sweetness | |
| | - Homoalanine-fermenting starters | *L. lactis* |
| | - Galactose-positive/glucose-negative starters | *Lb. delbrueckii* subsp. *bulgaricus, S. thermophilus* |
| | - malolactic fermentation | *O. oeni* |
| Nutritional | Production of nutraceuticals | |
| | - Low-calories sugars | *Lb. plantarum* |
| | - Production of oligosaccharides | *L. lactis* |
| | - Production of B-group vitamins | *L. lactis, S. thermophilus* |
| | Reduction of toxic and anti-nutritional compound | |
| | - Production of L(+)-lactic acid isomer | L(+)-lactic acid-producing strains |
| | - Removal of lactose and galactose | *S. thermophilus* |
| | - Removal of raffinose in soy | Several strains |
| Technological | Bacteriophage resistance | Several strains |
| | Prevention of overacidification in yoghurt | Lactose-negative *Lb. delbrueckii* subsp. *bulgaricus* |
| | Autolysing starters | |
| | - Phage-mediated | *L. lactis* subsp. *lactis* |
| | - Bacteriocin-induced | *L. lactis* |

[a] *E.=Enterococcus, L.=Lactococcus, Lb.=Lactobacillus, O.=Oenococcus, P.=Pediococcus, S.=Streptococcus*

The implementation of carefully selected strains as starter cultures or co-cultures in fermentation processes can help to achieve in situ expression of desired property, maintaining a perfectly natural and healthy product. Examples are lactic acid bacteria that are able to produce antimicrobial substances, sugar polymers, sweeteners, aromatic compound, useful enzymes, or nutraceuticals, or lactic acid bacteria with health-promoting properties, so called probiotic strains. This represents a way of replacing chemical additives by natural compounds, at the same time providing the consumer with new, attractive food products. It also leads to a wider application area and higher flexibility of starter cultures.

## CONCLUSION

Lactic acid bacteria display numerous antimicrobial activities in fermented foods. This is mainly due to the production of organic acids, but also of other compounds, such as ethanol, $H_2O_2$, diacetyl, reuterin and bacteriocins. Several bacteriocins with industrial potential have been purified and characterized. Application of bacteriocin-producing starter cultures in fermented foods has been studied during in vitro laboratory fermentations as well as on pilot-scale level. The promising results of these studies underline the important role that bacteriocinogenic lactic acid bacteria may play in food industry as starter cultures to improve food quality and safety.

## REFERENCES

[1] M.E. Stiles, W.H. Holzapfel, *Int. J. Food Microbiol.*, **1997**, 36, 1-29.
[2] L. De Vuyst, E.J. Vandamme, In: L. De Vuyst, E.J. Vandamme (Ed.), Bacteriocin of Lactic Acid Bacteria, (Blackie Academic & Professional, California, **1993**) 1-12.
[3] L. Axelsson, In: S. Salminen, A. von Wright (Ed.), Lactic Acid Bacteria: Microbiology and Functional Aspects, 2nd Edition, (Marcel Dekker Inc, New York, **1998**) 1-72.
[4] S.E. Gilliand, *FEMS Microbiol. Rev.*, **1990**, 87, 175-178.
[5] S.E. Lindgren, W.J. Dobrogosz, *FEMS Microbiol. Rev.*, **1990**, 87, 149-164.
[6] E. Caplice, G.F. Fitzgerald, *Int. J. Food Microbiol.*, **1999**, 50, 131-149.
[7] R.P. Ross, S. Morgan, C. Hill, *Int. J. Food Microbiol.*, **2002**, 79, 3-16.
[8] A.M. Daeschel, *Food Technol.*, **1989**, 43, 164-166.
[9] S. Codon, *FEMS Microbiol. Rev.*, **1987**, 269-280.
[10] L. Axelsson, T.C. Chung, W.J. Dobrogosz, *Microbiol. Ecol. Health Dis*., **1989**, 2, 131-136.
[11] M.G. Ganzle, A. Holtzel, J. Walter, G. Jung, W.P. Hammes, *Appl. Environ. Microbiol.*, **2000**, 66, 4325-4333.
[12] K.A. Stevens, B.W. Sheldon, *J. Food Prot.*, **1992**, 55, 763-766.
[13] R.W. Jack, J.R. Tagg, B. Ray, *Microbiol. Rev.*, **1995**, 59, 171-200.
[14] J.R. Tagg, A.S. Dajani, L.W. Wannamaker, *Bacteriol. Rev.*, **1976**, 40, 722-756.
[15] A. Hurst, *Adv. Appl. Microbiol.*, **1981**, 27, 85-123.
[16] B. Ray, *ASM News*, **1993**, 59, 285-291.
[17] T. Abee, L. Krockel, C. Hill, *Int. J. Food Microbiol.*, 1995, **28**, *169-185*.
[18] N. Kalchayanand, T. Sikes, C.P. Dunne, B. Ray, *Appl. Environ. Microbiol.*, **1994**, 60, 4174-4177.
[19] K.A. Stevens, B.W. Sheldon, N.A. Klapes, T.R. Klaenhammer, *Appl. Environ. Microbiol.*, **1992**, 57, 3613-3615.
[20] C.I. Mortvedt-Abildgaard, J. Nissen-Meyer, B. Jelle, B. Grenov, M. Skaugen, I.F. Nes, *Appl. Environ. Microbiol.*, **1995**, 61, 175-179.
[21] M.J. Garcia-Garcera, M.G.L. Elferink, A.J.M. Driessen, W.N. Koning, *Eur. J. Biochem.*, **1993**, 212, 417-422.
[22] U. Schillinger, F.K. Lucke, Appl. *Environ. Microbiol.*, **1989**, 55, 1901-1906.

[23] G.E. Allison, R.W. Worobo, M.E. Stiles, T.R. Klaenhammer, *Appl. Environ. Microbiol.*, **1995**, 61, 1371-1377.
[24] O.P. Kuipers, H.S. Rollema, W.M.G.J. Yap, H.J.Boot, R.J. Siezen, W.M. de Vos, *J. Biol. Chem.*, **1992**, 267, 24340-24346.
[25] T.R. Klaenhammer, *FEMS Microbiol. Rev.*, **1993**, 12, 39-86.
[26] I.F. Nes, D.B. Diep, L.S. Havarstein, M.B. Brurberg, V. Eijsink, H. Holo, *Antonie van Leeuwenhoek*, **1996**, 70, 113-128.
[27] R.W. Jack, J.R. Tagg, B. Ray, *Microbiol. Rev.*, **1995**, 59, 171-200.
[28] L. De Vuyst , E.J. Vandamme, In:. De Vuyst, E.J. Vandamme (Ed.), Bacteriocin of Lactic Acid Bacteria, (Blackie Academic & Professional, California **1993**) 152-221.
[29] R. Benkerroum, W.E. Sandine, *J. Dairy Sci.*, **1988**, 71, 3237-3245.
[30] L.J. Harris, H.P. Fleming, T.R. Klaenhammer, *Appl. Environ. Mcrobiol.*, **1992**, 58, 1484-1489.
[31] F. Breidt, K.A. Crowley, H.P. Fleming, *Appl. Environ. Mcrobiol.*, **1993**, 59, 3778-3783.
[32] S.Y. Choi, I.S. Lee, J.Y. Too, K.S. Chung, Y.J. Koo, *Korean J. Appl. Microbiol. Technol.*, **1990**, 18, 620-623.
[33] K. Rayman, N. Malik, A. Hurst, *Appl. Environ. Mcrobiol.*, **1983**, 46, 1450-1452.
[34] J. Hugenholtz, M. Twigt, M. Slomp, M.R. Smith, In: J. Hugenholtz, M. Twigt, M. Slomp, M.R. Smith (Ed.)**,** International Dairy Lactic Acid Bacteria Conference, 1995, (Palmerston North, New Zealand, **1995**) S. 2.4.
[35] O. McAuliffe, M.P. Ryan, P.R. Ross, C. Hill, P. Breeuwer, T. Abee, *Appl. Environ. Mcrobiol.*, **2000**, 4, 439-445.
[36] M.P. Ryan, M.C. Rea, C. Hill, R.P. Ross, *Appl. Environ. Microbiol.*, **1996**, 62, 612-619.
[37] C.F. Gonzalez, B.S. Kunka, *Appl. Environ. Mcrobiol.*, **1987**, 53, 2534-2538.
[38] H. Kimura, R. Nagano, H. Matsusaki, K. Sonomoto, A. Ishizaki, *Biosci. Biotechnol. Biochem*., **1997**; 61: 1049-1051.
[39] E.D. Berry, M.B. Liewen, R.M. Mandigo, R.W. Hutkins, *J. Food Prot*., **1990**, 53, 194-197.
[40] P.M. Foegeding, A.B. Thomas, D.H. Pilkington, T.R. Klaenhammer, *Appl. Envion. Microbiol.*, **1992**, 58, 884-890.
[41] M. Campanini, I. Pedrazzoni, S. Barbuti, P. Baldini, *Int. J. Food Microbiol.*, **1993**, 20, 169-175.
[42] A.L. Holck, L. Axelsson, K. Huhne, L. Krockel, *FEMS Microbiol. Let*., **1994**, 115, 143-150.
[43] M.C. Joerger, T.R. Klaenhammer, *J. Bacteriol*., **1986**, 167: 439-446.
[44] T. Nilsen, I.F. Nes, H. Holo, *Appl. Environ. Microbiol.*, **2003**, 69, 2975-2984.
[45] H. Oberman, Z. Libudzisz, In: B.J.B Wood (Ed.), Microbiology of Fermented Foods, (Blackie Academic & Professional, London, **1998**) 308-350.
[46] W.E. Sandine, In: T.M. Cogan, J.P. Accolas (Ed.), Dairy Starter Cultures, (Wiley-VCH, New York, **1996**) 191-206.
[47] T.P. Beresford, N.A. Fitzsimons, N.L. Brennan, T.M. Cogan, *Int. Dairy J.*, **2001**, 11, 259-274.
[48] E.M. Beukes, B.H. Bester, J.F. Mostert, *Int. J. Food Microbiol.*, **2001**, 63, 189-197.
[49] B.A. Law, *Int. Dairy J.*, **2001**, 11, 383-398.
[50] L. De Vuyst, *Food Technol. Biotechnol.*, **2000**, 38, 105-112.