Lactic Acid Bacteria (page 1)

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Lactic Acid Bacteria

*Lactobacillus acidophilus* The bacterium is a member of the normal flora of humans, found in the oral cavity, the small intestine, and the vaginal epithelium, where it is thought to play a beneficial role. The organism is generally the first bacterium listed as present in probiotic concoctions.

Lactic Acid Bacteria (LAB) are Gram-positive, non-sporeforming cocci, coccobacilli or rods with a DNA base composition of less than 53 mol% G+C. They generally are non respiratory and lack catalase. They ferment glucose primarily to lactic acid, or to lactic acid, CO₂ and ethanol. All LAB grow anaerobically, but unlike most anaerobes, they grow in the presence of O₂ as "aerotolerant anaerobes". Although they lack catalase, they possess superoxide dismutase and have alternative means to detoxify peroxide radicals, generally through peroxidase enzymes.

Although many genera of bacteria produce lactic acid as a primary or secondary end-product of fermentation, the term Lactic Acid Bacteria is conventionally reserved for genera in the order Lactobacillales, which includes *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*, in addition to *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weisella*.

Because they obtain energy only from the metabolism of sugars, lactic acid bacteria are restricted to environments in which sugars are present. They have limited biosynthetic ability, having evolved in environments that are rich in amino acids, vitamins, purines and pyrimidines, so they must be cultivated in complex media that fulfill all their nutritional requirements. Most are free-living or live in beneficial or harmless associations with animals, although some are opportunistic pathogens. They are found in milk and milk products and in decaying plant materials. They are normal flora of humans in the oral cavity, the intestinal tract and the vagina, where they play a beneficial role.

A few LAB are pathogenic for animals, most notably some members of the genus *Streptococcus*. In humans, *Streptococcus pyogenes* is a major cause of disease (strept throat, pneumonia, and other pyogenic infections, scarlet fever and other toxemias). *Streptococcus pneumoniae* causes lobar pneumonia, otitis media and meningitis; some viridans and nonhemolytic oral streptococci play a role in dental caries and may be an insidious cause of endocarditis. The pathogenic streptococci are dealt with elsewhere in the text. This chapter deals primarily with LAB in association with food and dairy microbiology, to a lesser extent with LAB as beneficial components of the human normal flora and probiotics.

Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. They contribute to the taste and texture of fermented products and inhibit food.
Spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid. As agents of fermentation LAB are involved in making yogurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives and sauerkraut, but some species may spoil beer, wine and processed meats.

Differential characteristics of lactic acid bacteria based on morphology and physiology

<table>
<thead>
<tr>
<th>Genus</th>
<th>Lactobacillus</th>
<th>Enterococcus</th>
<th>Lactococcus</th>
<th>Leuconostoc</th>
<th>Pediococcus</th>
<th>Streptococcus</th>
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<tr>
<td>Morphology</td>
<td>rods</td>
<td>cocci</td>
<td>cocci</td>
<td>cocci</td>
<td>cocci in tetrads</td>
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<tr>
<td>CO₂ from glucose</td>
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<td>−−</td>
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<td>−−</td>
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<tr>
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<td>L, DL</td>
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<td></td>
</tr>
</tbody>
</table>

*test for homo- or heterofermentation of glucose: - homofermentation + heterofermentation

+ positive; - negative; ± varies between species

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Lactic Acid Bacteria (page 2)

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Metabolism

The essential feature of LAB metabolism is efficient carbohydrate fermentation coupled to substrate-level phosphorylation. Adenosine triphosphate (ATP) generated is subsequently used for biosynthesis. LAB as a group exhibit an enormous capacity to degrade different carbohydrates and related compounds. Generally, the predominant end product is lactic acid (>50% of sugar carbon). However, LAB adapt to various conditions and change their metabolism accordingly. This may lead to significantly different end-product patterns.

Based on sugar fermentation patterns, two broad metabolic categories of LAB exist: homolactic and heterolactic. The first category, homolactic LAB, includes some lactobacilli and most species of lactococci, lactosoci, pediococci, streptococci, tetragenococci, and vagococci, that ferment hexoses by the Embden-Meyerhof (E-M) pathway. The second category, heterolactic LAB, includes leuconostocs, some lactobacilli, oenococci, and weissela species. The apparent difference on the enzyme level between these two categories is the presence or absence of the key cleavage enzymes of the E-M pathway (fructose 1,6-diphosphate) and the PK pathway (phosphoketolase).

Homolactic Fermentation

Under conditions of excess glucose and limited oxygen, homolactic LAB catabolize one mole of glucose in the Embden-Meyerhof pathway to yield two moles of pyruvate. Intracellular redox balance is maintained through the oxidation of NADH, concomitant with pyruvate reduction to lactic acid. This process yields two moles of ATP per glucose consumed. Representative homolactic LAB genera include Lactobacillus, Lactococcus, Enterococcus, Streptococcus, and Pediococcus species.

The transport and phosphorylation of sugars occur by (1) transport of free glucose and phosphorylation by an ATP-dependent hexose kinase (other sugars, such as mannose and fructose, enter the major pathways at the level of glucose-6-phosphate or fructose-6-phosphate after isomerization or phosphorylation or both); or (2) the phosphoenolpyruvate (PEP) sugar phosphotransferase system (PTS), in which PEP is the phosphoryl donor for the uptake of sugar. Some species of LAB use the PTS for transport of galactose only; others use the PTS for all sugars.
The pathway of homolactic acid fermentation in Lactic Acid Bacteria

**Heterolactic Fermentation**

Heterofermentative LAB utilize the phosphoketolase pathway (pentose phosphate pathway) to dissiplate sugars. One mole of glucose-6-phosphate is initially dehydrogenated to 6-phosphogluconate and subsequently decarboxylated to yield one mole of CO₂. The resulting pentose-5-phosphate is cleaved into one mole glyceraldehyde phosphate (GAP) and one mole acetyl phosphate. GAP is further metabolized to lactate as in homofermentation, with the acetyl phosphate reduced to ethanol via acetyl-CoA and acetaldehyde intermediates. Theoretically, end-products (CO₂, lactate and ethanol) are produced in equimolar quantities from the catabolism of one mole of glucose. Obligate heterofermentative LAB include Leuconostoc, Oenococcus, Weissella, and certain lactobacilli.
The pathway of heterolactic acid fermentation in Lactic Acid Bacteria

Lactic acid bacteria have a very limited capacity to synthesize amino acids using inorganic nitrogen sources. They are therefore dependent on preformed amino acids being present in the growth medium as a source of nitrogen. The requirement for amino acids differs among species and strains within species. Some strains are prototrophic for most amino acids, whereas others may require 13–15 amino acids. Since the quantities of free amino acids present in their environment are not sufficient to support the growth of bacteria to a high cell density, they require a proteolytic system capable of hydrolyzing peptides and proteins in order to obtain essential amino acids. All dairy lactococci used for acidification of milk (e.g., in cheese manufacture) have proteolytic activity. The lactococcal proteolytic system consists of enzymes outside the cytoplasmic membrane, transport systems, and intracellular peptidases. The proteolytic activity of LAB contributes additionally to the development of the flavor, aroma and texture of fermented products. For many varieties of cheeses, such as Swiss and Cheddar, desirable “flavor tones” are derived by proteolysis.
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Fermentation of Foods by Lactic Acid Bacteria

Many human foods are plants or animal products which have been fermented by lactic acid bacteria, since these bacteria possess properties that can benefit food production or conversion. The acidic and organoleptic properties of fermented foods result from the metabolic activities of these microorganisms. Foods such as ripened cheeses, fermented sausages, sauerkraut and pickles have not only a greatly extended shelf life compared to the raw materials from which they are derived, but also aroma and flavor characteristics contributed directly or indirectly by the fermenting organisms.

Fermented dairy products have been made for thousands of years, but only within the last century have the microbiological bases of these fermentations been elucidated. Lactic acid bacteria are the principal organisms involved in fermenting dairy products. Prior to the availability of starter cultures, milk fermentations relied on the LAB naturally present in raw milk. The first commercial starter cultures were unknown mixes of microbes from raw milk that were prepared in Denmark around end of the 19th century. In the 1930s and 40s, the idea of pure single-strain starter cultures evolved.

Fermented dairy products are enjoying increased popularity as convenient, nutritious, stable, natural, and healthy foods. Lactic acid bacteria are the principal organisms involved in the manufacture of cheese, yogurt, buttermilk, cottage cheese, sour cream and cultured butter. In some fermented dairy products, additional bacteria, referred to as secondary microflora, are added to produce carbon dioxide, which influences the flavor and alters the texture of the final product.

Sausage is one of the oldest processed meat products. The writings of ancient Egyptians described the preservation of meat by salting and sun drying. The ancient Babylonians, Greeks, and Romans used sausage as a food source during times of war. Microorganisms were recognized as being important to the production of sausages about 1921. In the 1940s and 1950s, pure microbial starter cultures consisting of lactic acid bacteria became available but their use was not widespread until the early 1980s.

The fermentation of vegetables, a practice that originated in the Orient, has been used as a means of preserving food for more than 2,000 years. In the third century B.C., during the construction of the Great Wall of China, the Chinese produced fermented vegetables (cabbages, radishes, turnips, cucumbers, etc.) on a large scale. The most common fermented vegetables available in the United States are pickles, sauerkraut, and olives. Carrots, cauliflower, celery, okra, onions, and sweet and hot peppers also are sold as fermented vegetable products.

Generally LAB that are important in the fermentation of food products (dairy, meat, vegetables, fruits, and beverages), include only certain species of the genera Lactobacillus, Lactococcus, Streptococcus, Leuconostoc and Pediococcus. Some of these species are also members of normal flora of the mouth, intestine, and vagina of mammals.

<table>
<thead>
<tr>
<th>Involvement of lactic acid bacteria in the manufacture of fermented dairy products</th>
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<tbody>
<tr>
<td><strong>Product</strong></td>
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</tr>
<tr>
<td>Blue</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Lactococcus lactis subsp. cremoris</td>
</tr>
<tr>
<td>Mozzarella, provolone, Romano, parmesan</td>
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<td></td>
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<tr>
<td>Buttermilk</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Sour cream</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Lactobacilli**

Lactobacilli is a very heterogeneous genus, encompassing species with a large variety of phenotypic, biochemical, and physiological properties. Most species of lactobacilli are homofermentative, but some are heterofermentative. The genus has been divided into three major subgroups and over 70 species are recognized.

Group I lactobacilli are obligately homofermentative and produce lactic acid as a major end product (>85%) from glucose. They are represented by *L. delbrueckii* and *L. acidophilus*. They grow at 45°C but not at 15°C. Group II, also homofermentative, grow at 15°C and show variable growth at 45°C. Represented by *L. casei* and *L. plantarum*, they can produce more oxidized fermentations (e.g. acetate) if O2 is present. Group III lactobacilli are heterofermentative. They produce lactic acid from glucose, along with CO2 and ethanol. Aldolase is absent and phosphoketolase is present. Representative species include *L. fermentum*, *L. brevis* and *L. kefiri*.

Lactobacilli are often found in dairy products, and some species are used in the preparation of fermented milk products. For example *L. delbrueckii* subsp. bulgaricus are used in the preparation of yogurt; *L. acidophilus* is used in the preparation of acidophilus milk; *L. helveticus*, as well as *L. delbrueckii* subsp. bulgaricus, are used to make Swiss, Mozzarella, provolone, Romano, and parmesan cheeses. Other species are used in the production of sauerkraut, silage and pickles. The lactobacilli are usually more resistant to acidic conditions than are other LAB, being able to grow at pH values as low as 4. This enables them to continue to grow during natural lactic fermentations when the pH has dropped too low for other LAB to grow, so they are often responsible for the final stages of many lactic acid fermentations.

Many sausage fermentations include a Lactobacillus species. *L. plantarum* is used in starter cultures for the manufacture of summer sausage, pepperoni and salami.

The natural fermentation of cabbage to make sauerkraut involves *L. brevis* and *L. plantarum* in the final succession of microbes. The starter cultures for manufacture of cucumber pickles includes *L. brevis* and *L. plantarum*. *L. plantarum* is the most essential species in pickle production, as it is for sauerkraut. Like cucumbers, olives are fermented under conditions similar to those of other vegetable products. The microbial population responsible for the fermentation of olives differs from that of sauerkraut and pickles mainly because the higher salt concentration of the brine prevents many salt-sensitive strains from growing and provides an advantage to salt-tolerant strains. LAB become prominent during the intermediate stage of fermentation. *L. mesenteroides* and *P. cerevisiae* are the first lactic to become predominant, followed by lactobacilli, with *L.
plantarum and L. brevis being the most important.

Streptococci and Lactococci

Note on Streptococcal Classification. Since 1985, members of the diverse genus Streptococcus have been reclassified into Lactococcus, Vagococcus, Enterococcus, and Streptococcus, based on biochemical characteristics, as well as ssRNA analysis. Historically, streptococci were segregated into serological groups based on the presence of specific carbohydrate antigens. Antigenic groups, or Lancefield groups (named for Rebecca Lancefield, a pioneer in Streptococcus taxonomy), are designated by letters A through O. Lancefield groups have proven to correlate well with the current taxonomic definitions. The beta-hemolytic streptococci found in humans contain the group A antigen, while “fecal streptococci” (enterococci) contain the group D antigen. Group B streptococcci, usually found in animals, are a cause of mastitis in cows, and have been implicated in human infections. “Lactic streptococci” (streptococci and lactococci) contain the group N antigen and are nonpathogenic. However, Lactococcus has been defined as a genus separate from Streptococcus. As lactic acid bacteria, Lactococcus lactis and Streptococcus thermophilus are the cornerstones of cheese manufacture.

Streptococcus thermophilus

Streptococcus thermophilus is an alpha-hemolytic species of the viridans group. The bacterium is found in milk and milk products. It is not a probiotic (it does not survive the stomach) and generally is used in the production of yogurt and the manufacture of several types of cheese, especially Italian and Swiss cheeses. The organism is a moderate thermophile with an optimal growth rate at 45 °C. Although S. thermophilus is closely related to other pathogenic streptococci (such as S. pneumoniae and S. pyogenes), S. thermophilus is classified as a non-pathogenic, alpha-hemolytic species that is part of the viridans group. It is closely related to S. salivarius in the oral cavity.

Lactococcus lactis

Lactococcus is a genus of LAB with five major species formerly classified as Group N streptococci. The type species for the genus is L. lactis, which has two subspecies, lactis and cremoris. Lactococci differ from other lactic acid bacteria by their pH, salt and temperature tolerances for growth.

Lactococcus lactis is critical for manufacturing cheeses such as Cheddar, cottage, cream, Camembert, Roquefort and Brie, as well as other dairy products like cultured butter, buttermilk, sour cream and kefir. The bacterium can be used in single strain starter cultures, or in mixed strain cultures with other lactic acid bacteria such as Lactobacillus and Streptococcus.
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Bacteriocins and LAB

Class I bacteriocins or lantibiotics are small peptides containing the unusual dehydroamino acids and thioether amino acids lanthionin and 3-methylanthionine, which are synthesized by Gram-positive bacteria during posttranslational modifications. These peptides are thought to attach to the membrane of target cells and, by an as yet unknown conformational rearrangement, lead to increased permeability and disruption of the membrane potential. There are two types of lantibiotics, types A and B. The lantibiotics produced by LAB all belong to type A, which are elongated screw-shaped peptides, whereas type B lantibiotics are mainly globular. Nisin produced by Lactococcus lactis ssp. lactis has been studied extensively. It has a broad spectrum of activity against Gram-positive bacteria. The primary target is believed to be the cell membrane. Unlike some other antimicrobial peptides, nisin does not need a receptor for its interaction with the cell membrane; however, the presence of a membrane potential is required.

Starter cultures

Starter cultures consisting of lactic acid bacteria are added at the beginning of the cheesemaking process. Lactic acid bacteria are essential for manufacture of cheese, yogurt, sour cream, cultured butter and most fermented milk products.

Starter cultures play an essential part in the manufacture of fermented dairy products. They produce the lactic acid that coagulates milk and they contribute to texture, moisture content, freedom from pathogenic microorganism, and taste of the product. The rate of acid production is critical in the manufacture of certain products, e.g., Cheddar cheese. Depending on the product, especially in mechanized cheese production units, starters may also be required to produce acid at a consistently fast rate through the manufacturing period each day and every day. The negative redox potential created by starter growth in cheese also aids in preservation and the development of flavor in Cheddar and similar cheeses. Additionally, antibiotic substances, now referred to as bacteriocins, produced by starters, e.g., nisin, may also have a role in preservation.

Ecology of starter bacteria

Most starters in use today have their origins as lactic acid bacteria naturally present as part of the contaminating microflora of milk. These bacteria probably came from vegetation in the case of lacticocci or the intestinal tract in the case of bifidobacteria, enterococci and Lactobacillus acidophilus.

Modern starter cultures developed from the practice of retaining small quantities of whey or cream from the successful manufacture of a fermented product on a previous day and using this as the inoculum or starter for the following day’s production. In the foods
industry his practice has been referred to as "back-slopping".

Classification of starter cultures
While the microbes used in the manufacture of fermented dairy products are generally lactic acid bacteria, Propionibacterium shermanii and Bifidobacterium spp. which are not lactic acid bacteria, are also used. In addition, other bacteria including trehalobacterium (which is responsible for the flavor of Limburger cheese) and molds (Penicillium species) are used in the manufacture of Camembert, Roquefort and blue cheeses.

Probiotics and Lactic Acid Bacteria
Probiotics are products designed to deliver potentially beneficial bacterial cells to the microbiotic ecosystem of humans and other animals. Strains of lactic acid bacteria are the most common microbes employed as probiotics, especially Lactobacillus and Bifidobacterium species, but lactococci, some enterococci and some streptococci are also included as probiotics.

Lactic acid-producing Bacteria Used as Probiotics

**Lactobacillus**
Lactobacillus species are facultative anaerobes. They grow in the presence of O2, however, and may convert it to H2O or H2O2. Lactobacilli normally predominate in the small intestine, and they are known for their beneficial effects which may antagonize potential pathogens. Of the more than 100 Lactobacillus species, the following are commonly used probiotics:
- L. acidophilus
- L. fermentum
- L. paracasei
- L. brevis
- L. gasseri
- L. plantarum
- L. bulgaricus
- L. helveticus
- L. reuteri
- L. casei
- L. jenseni
- L. rhamnosus
- L. crispatus
- L. johnsonii
- L. salivarius

**Bifidobacterium**
Bifidobacteria are strictly anaerobic and normally vie for predominance in the large intestine. Among 30 species, those recognized as probiotics include:
- B. adolescentis
- B. breve
- B. longum
- B. animalis
- B. infantis
- B. thermophilum
- B. bifidum
- B. lactis

Bifidobacterium longum: Bifidobacteria are an obligately anaerobic bacteria, not classified with the lactic acid bacteria, but which occupy similar habitats and produce lactic acid as a sole end-product. They are a prominent Gram-positive bacterium in the large intestine (colon). Bifidobacterium infantis is the predominant bacterium in the intestine of breast-fed infants because mother's milk contains a specific growth factor that enriches for the growth of the
**Streptococcus**

Streptococcus species are not typically associated with health benefits and some are highly pathogenic. However, one facultative anaerobic species, *Streptococcus thermophilus*, is known to promote health. It is one of the two primary species found in yogurt cultures, the other being *L. bulgaricus*.

**Enterococcus**

Found in a number of probiotic products, the facultative anaerobe *Enterococcus faecium* is invariably a component of the normal intestinal microbiota and is considered a beneficial microbe. However, *E. faecium* has evolved from a relatively nonpathogenic commensal bacterium to the third most common cause of hospital-acquired infections and now accounts for over 10% of enterococcal clinical isolates. Furthermore, it has developed extensive resistance to antibiotics, which it is capable of transferring to other bacteria.

The human body, primarily the gastrointestinal tract, is home to a large number of different species of bacteria, and it is likely we could not survive without their presence. Two of the most common bacteria that comprise the intestinal microbiota (“normal flora”) are *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Hence, they are a main component of probiotics.

Enterococcus faecium is invariably a component of the normal intestinal microbiota and is considered a beneficial microbe. However, *E. faecium* has evolved from a relatively nonpathogenic commensal bacterium to the third most common cause of hospital-acquired infections and now accounts for over 10% of enterococcal clinical isolates. Furthermore, it has developed extensive resistance to antibiotics, which it is capable of transferring to other bacteria.

The indigenous bacteria of humans serve a wide range of functions, which include manufacture of some B vitamins and vitamin K, synthesis of some digestive enzymes (e.g., lactase), competition with pathogens for colonization sites, production of antibacterial and antifungal substances that protect against harmful organisms, production of chemicals that have been shown to be anti-carcinogenic, and stimulation of the development and activity of the immune system.

The natural balance of the body's bacteria can be upset by several factors such as certain medicines, antibiotics and steroids, increased acidity in the digestive system caused by stress, lack of sleep and poor diet, constipation or diarrhea, yeast overgrowth, fatigue, IBS and other intestinal conditions.

It has been suggested, in a few cases proven, that one way to combat these conditions is by supplementation of the diet with probiotic bacteria in natural foods or artificial supplements.

Probiotics have been recommended or suggested for patients receiving radiation treatment, individuals who have recurrent thrush, vaginal yeast infections, or urinary tract infections, persons suffering from irritable bowel syndrome (IBS) or other bowel problems, for travelers abroad to protect against food poisoning and during any period where antibiotics may be taken.

**L. Plantarum**

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Lactic Acid Bacteria (page 5)

The following section that focuses on Lactococcus lactis as the prototypical lactic acid bacterium. *Lactococcus lactis* exhibits virtually all of the foregoing characteristics and applications of a lactic acid bacterium. This bacterium is chosen because of its preeminence in cheese making and its status as the natural nominee as the state microbe of the dairy state of Wisconsin.

*Lactococcus lactis*. UW Department of Bacteriology strain LcL325UW. Magnification 20000X. Scanning electron micrograph by Joseph A. Heintz, University of Wisconsin-Madison.

*Lactococcus lactis* is a microbe classified informally as a Lactic Acid Bacterium because it ferments milk sugar (lactose) to lactic acid. Lactococci are typically spherical or ovoid cells, about 1.2 µm by 1.5 µm, occurring in pairs and short chains. They are Gram-positive, non motile, and do not form spores. Lactococci are found associated with plant material, mainly grasses, from which they are easily inoculated into milk. Hence, they are found normally in milk and may be a natural cause of souring. *Lactococcus lactis* has two subspecies, *lactis* and *cremoris*, both of which are essential in manufacture of many varieties of cheese and other fermented milk products.

*Lactococcus lactis* is related to other lactic acid bacteria such as *Lactobacillus acidophilus* in our intestinal tract and *Streptococcus salivarius* in the mouth. However, *Lactococcus* does not normally colonize human tissues and differs from many other lactic acid bacteria in its pH, salt, and temperature tolerances for growth, which are important characteristics relevant to its use as a starter culture in the cheesemaking industry.

*Lactococcus lactis* is vital for manufacturing cheeses such as Cheddar, Colby, cottage cheese, Camembert, Roquefort and Brie, as well as other dairy products like cultured butter, buttermilk, sour cream and kefir. It may also be used for vegetable fermentations such as cucumber pickles and sauerkraut. The bacterium can be used in single strain starter cultures, or in mixed strain cultures with other lactic acid bacteria such as *Lactobacillus* and *Streptococcus* species.

When *Lactococcus lactis* is added to milk, the bacterium uses enzymes to produce energy (ATP) from lactose. The byproduct of ATP production is lactic acid. The lactic acid curdles
the milk that then separates to form curds, which are used to produce cheese and whey.
Curdling the milk is not the bacterium's only role in cheese production. The lactic acid produced by the bacterium lowers the pH of the product and preserves it from the growth by unwanted bacteria and molds while other metabolic products and enzymes produced by Lactococcus lactis contribute to the more subtle aromas and flavors that distinguish different cheeses.

Fermented dairy products wherein Lactococcus lactis is the primary organism involved in manufacture.

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<th>Principal acid producers</th>
<th>Secondary microflora</th>
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Lactococcus lactis. Magnification 1500X. Phase micrograph courtesy of T.D. Brock, University of Wisconsin-Madison.

Cheese

Cheese making is essentially a dehydration process in which milk casein, fat and minerals are concentrated 6 to 12-fold, depending on the variety. The basic steps common to most varieties are acidification, coagulation, dehydration, and salting. Acid production is the major function of the starter bacteria. Lactic acid is responsible for the fresh acidic flavor of unripened cheese and is important in coagulation of milk casein, which is accomplished by the combined action of rennet (an enzyme) and lactic acid produced by the microbes. During the ripening process the bacteria play other essential roles by producing volatile flavor compounds (e.g., diacetyl, aldehydes), by releasing proteolytic and lipolytic enzymes involved in cheese ripening, and by producing natural antibiotic substances that suppress growth of pathogens and other spoilage microorganisms. For Cheddar and Colby cheese production, starter cultures include strains of Lactococcus lactis ssp. cremoris and/or lactis. Likewise, blue cheeses require Lactococcus lactis ssp. cremoris or lactis, but the mold Penicillium roqueforti is also added as a secondary culture for flavor and blue appearance.
Wisconsin’s unique cheese curds, Colby, and dozens of varieties of Cheddar are made exclusively with strains of Lactococcus lactis. Images courtesy of Wisconsin Cheese Mart, Milwaukee Wisconsin.

Cultured Butter, Buttermilk and Sour Cream

Sour cream is made from cream to which a starter culture of Lactococcus lactis has been added to coagulate the cream and to enhance its flavor. Buttermilk is also made with Lactococcus lactis in order to acidify, preserve and flavor the milk. Diacetyl, made from citrate by Lactococcus, gives buttermilk its distinct taste and enhances its storage properties. Lactococcus lactis or mixed cultures that contain Lactococcus lactis, plus a Leuconostoc species are used. In the making of cultured butter, fat (cream) is separated from skim milk by centrifugation of milk. The cream is pasteurized and inoculated with selected starter cultures. The ripened cream is then churned. The cream separates again into cream butter and its byproduct, sour buttermilk.

Nisin

Nisin is an antibiotic-like substance, called a bacteriocin, produced by the “food grade” starter strain, Lactococcus lactis ssp. lactis. It is a natural antimicrobial agent with activity against a wide variety of Gram-positive bacteria, including food-borne pathogens such as Listeria, Staphylococcus and Clostridium. The primary target of nisin is believed to be the cell membrane. Unlike some other antimicrobial peptides, nisin does not need a receptor for its interaction with the cell membrane; however, the presence of a membrane potential is required. Nisin is a natural preservative present in cheese made with Lactococcus lactis ssp. lactis, but it is also used as a preservative in heat processed and low pH foods. Since nisin cannot be synthesized chemically, the nisin-producing Lactococcus lactis strains are used for its industrial synthesis.

The first established use of nisin was as a preservative in processed cheese products, but numerous other applications in preservation of foods and beverages have been identified. It is currently recognized as a safe food preservative in approximately 50 countries. Nisin has been used as a preservative in various pasteurized dairy products and canned vegetables, baked, high-moisture flour products, and pasteurized liquid eggs. There is interest in the use of nisin in natural cheese production. Considerable research has been carried out on the anti-listerial properties of nisin in foods and a number of applications have been proposed. Uses of nisin to control spoilage lactic acid bacteria have been identified in beer, wine, alcohol production, and high acid foods such as salad dressings. Production of highly purified nisin preparations has led to interest in the use of nisin for human ulcer therapy and mastitis control in cattle.
Starter Cultures

Starter cultures have crucial roles to play during all phases of the cheese making and maturation process. As the culture grows in the milk, it converts lactose to lactic acid. This ensures the correct pH for coagulation and influences the final moisture content of the product. The rate of acid production is critical in the manufacture of certain products, e.g., Cheddar cheese. In mechanized operations, starters are often required to produce acid at a consistently fast rate through the manufacturing period each and every day. During ripening, culture, lipolytic and proteolytic enzymes are released from the bacteria that add a balanced aroma, taste, texture, and surface appearance to the product. The negative redox potential created by starter growth in cheese also aids in preservation and the development of flavor in Cheddar and similar cheeses. Additionally, antibiotic-like substances produced by starters (e.g., nisin) may also have a role in preservation.

Lactococcus and Vaccine Delivery

A recently discovered application of Lactococcus lactis is in the development of vaccine delivery systems. The bacterium can be genetically engineered to produce proteins from pathogenic species on their cell surfaces. Intra nasal inoculation of an animal with the modified strain will elicit an immune response to the cloned protein and provide immunity to the pathogen. For example, if one wished to provide immunity to Streptococcus pyogenes, the causative agent of strep throat, Lactococcus could be engineered to present the conserved portion of the streptococcal M protein required for streptococcal adherence and colonization to the nasopharyngeal mucosa. The resulting local immune response could protect the individual from strep throat caused by the streptococcus that exhibits that form of the M protein. This approach theoretically can be adapted to any pathogen that colonizes and/or enters via a mucosal surface in humans or animals. This includes human pathogens such as Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria meningitidis, Mycobacterium tuberculosis, Bordetella pertussis and Neisseria meningitidis, among others.

More than 4 million deaths per year are due to respiratory diseases. Economical and effective vaccines against respiratory pathogens are needed for implementation in poorer countries where the disease burden is highest. Following respiratory tract infection, some pathogens may also invade the epithelial tissues, achieving systemic circulation and spread to other organs. Nasal administration of different antigen formulations using Lactococcus lactis as a delivery vehicle has shown promising results in the induction of immune
Lactococcus lactis has been shown to deliver antigens that stimulate mucosal immunity to nonrespiratory pathogens, as well, including HIV, Human papilloma virus and the malarial parasite.

Some of the research papers that have employed Lactococcus lactis as a vector for vaccine delivery are cited below.


The Lactococcus Genome

Partly due to their industrial relevance, both Lactococcus lactis subspecies (lactis and cremoris) are widely used as generic LAB models for research. L. lactis ssp. cremoris, used in the production of hard cheeses, is represented by the laboratory strains LM9230 and MG1363. Similarly, L. lactis ssp. lactis is employed in soft cheese fermentations, with the workhorse strain IL1403, ubiquitous in LAB research laboratories. In 2001, the genome of strain IL1403 was sequenced leading to increased understanding of LAB genomics and related applications. Currently, there are two L. lactis ssp. cremoris that have been sequenced for public release.

A French group sequenced the genome of Lactococcus lactis ssp. lactis. The genome sequence reveals 12 enzymes called aminotransferases, some of which are used to break down complex, branched, ring-shaped, and sulfur-containing amino acids. The molecules produced when the amino acids are degraded are very important for cheese flavor. Understanding which amino acids are broken down by which enzymes could give cheese makers greater control over flavor and fragrance of their fromage.
Genome atlas of the chromosome of *L. lactis* MG1363. ifr.ac.uk

The sequence also led to the identification of 29 genes that are required to build the mesh-like peptidoglycan component of the bacterium’s cell wall. Inducing some of these enzymes can accelerate the slow, expensive process of cheese ripening, during which the cheese ages and develops its characteristic flavor. Learning how to selectively activate some of these enzymes could revolutionize the cheese manufacturing process.

The *Lactococcus lactis* ssp. *lactis* genome has 2,365,589 units (bp) of DNA, which contain 2,310 predicted genes. About 64 percent of the genes have assigned roles in the cell, while 20 percent match other hypothetical genes with unknown function. Almost 16 percent of the genes bear no resemblance to genes from other species and are considered to be unique to this bacterium.

The *L. lactis* genome contains six prophages (carrying nearly 300 genes or about 14 percent of the total coding capacity) and 43 insertion elements. Sequence data also revealed new possibilities for fermentation pathways and confirmed the total lack of genes and enzymes involved in the TCA cycle although, unexpectedly, certain genes necessary for aerobic respiration were found encoded in the genome.

It is anticipated that understanding the physiology and genetic make-up of this bacterium will prove invaluable for food manufacturers as well as the pharmaceutical industry, which is exploring the capacity of *L. lactis* to serve as a vehicle for delivering drugs and vaccines.

For example, genomic analysis of *Lactococcus lactis* ssp. *lactis* revealed the presence of several genes that encode enzymes involved in the reduction of pyruvate to various end-product other than homolactic acid, including ethanol, acetic acid, formic acid, diacetyl, acetoin and butanediol. Manipulation and understanding of the regulation of these genes could defeat or enhance the synthesis of these end products leading to strain improvement in starter cultures for cheeses, buttermilk, and other dairy products.