Porubcan RS and Sellars RL (1979) Lactic Starter Culture Concentrates. In: Peppler HJ and Perlman D (eds) *Microbial Technology. Microbial Processes*. P. 59. New York: Academic Press.

Prescott LM, Harley JP and Klein DA (1990) *Microbiology*, 2nd edn. DuBuque, USA: Wm. C. Brown Communications.

Steinkraus KH, Cullen RE and Gavitt BK (1983) *Handbook of Indigenous Fermented Foods*. New York: Marcel Dekker.

Tamine AY (1981) Microbiology of Starter Cultures. In: Robinson RK (ed.) *Dairy Microbiology*. P. 113. New York: Pergamon Press.

# *Lactobacillus brevis*

**Paula C M Teixeira**, Escola Superior de Biotecnologia, Porto, Portugal



## Characteristics of *Lactobacillus brevis*

*Lactobacillus brevis* is a microaerophilic, obligately heterofermentative lactic acid bacterium (uses the phosphoketolase pathway to produce a mixture of lactic acid, ethanol, acetic acid and $CO_2$ as products of hexose fermentation) which has been reported to lack phosphotransferase systems specific for glucose, fructose and lactose. Recent studies, however, demonstrated that anaerobic growth of *L. brevis* in the presence of fructose induces the synthesis of a phosphotransferase system and glycolytic enzymes that allow fructose to be metabolized via the Embden–Meyerhof pathway.

*L. brevis* is included in the second phylogenetic group of the lactobacilli, the *L. casei–Pediococcus* group.

*L. brevis* is normally isolated from milk, cheese, plants, sewage, cereal products, silage, fermented vegetables, fermented meats, cow manure, faeces, mouth and intestinal tract of humans and rats.

Carbohydrates fermented by *L. brevis* (90% or more strains) are arabinose, fructose, glucose, gluconate, maltose, melibiose and ribose. Esculin, galactose, lactose, raffinose, sucrose and xylose are fermented by 11–89% of the strains. *L. brevis* is unable to grow in chemically defined media having pentoses as a sole source of fermentable sugar. Identification of *L. brevis* strains by carbohydrate fermentation reactions or additional simple phenotypic tests has proved to be insufficient. Some strains earlier assigned to *L. brevis* have been assigned to new species on the basis of nucleic acid and biochemical data. In addition to DNA studies, electrophoretic mobility of lactic acid dehydrogenases is recommended to clearly distinguish *L. brevis* from *L. buchneri*, *L. bilgardii*, *L. collinoides* or *L. kefir* since some DNA of *L. brevis* strains hybridizes with that of some of these other lactobacilli.

Calcium pantothenate, niacin, thiamin and folic acid are essential growth factors but riboflavin, pyridoxal, and vitamin $B_{12}$ are not required.

*L. brevis* is considered to be a weakly proteolytic species.

Haem-independent nitrite reductases and haematin-requiring catalase activity have been found in *L. brevis*.

Cells are rod shaped with rounded ends, generally short and straight (0.7–1.0 × 2.0–4.0 μm). Long rods, however, are always present. They are usually separate or in short chains. Bipolar or other internal granulations are observed with the Gram reaction or methylene blue stain especially when cells become older. Most *L. brevis* strains possess immunologically heterogeneous S-layer proteins with molecular weights in the range 38–55 kDa.

Colonies are generally rough or intermediate, flat, and they may be nearly translucent. Although some strains are pigmented orange to red, they are generally non-pigmented. Additional physiological and biochemical characteristics are presented in **Table 1**.

Fatty acid composition has been used in the grouping and classification of microorganisms. As shown in **Table 2**, hexadecanoic acid (16:0), octadecenoic acid (18:1) and lactobacillic acid (19:0) are the major fatty acids present in *L. brevis*. However, there is variability at different stages of growth, between strains, as a result of different growth conditions (medium composition, temperature), and if different methodologies are used for lipids extraction.

Antibiotic resistance in lactic acid bacteria has been studied as a potential means of identification. However, no definite patterns of resistance have emerged to allow for use in a classification scheme.

**Table 1** Physiological and biochemical characteristics of *Lactobacillus brevis*

| | |
|---|---|
| G+C content (mol%) | 44–47 |
| Peptidoglycan type | Lys-D-Asp |
| Techoic acid | Glycerol |
| Antigenic group | E |
| Lactic acid isomer | DL |
| **Electrophoretic mobility** | |
| D-*LDH*[a] | 1.62 |
| L-*LDH* | 1.40 |
| Optimum pH | 4.0–5.0 |
| **Growth temp.** | |
| *Optimum* (°C) | 30 |
| *Minimum* (°C) | 2–4 |
| $NH_3$ from arginine | + |

[a]LDH, lactate dehydrogenase.

**Table 2** Major fatty acid components of lipids from *Lactobacillus brevis* strains determined by gas–liquid chromatography

| L. brevis *strain* | *14:0* | *15:0* | *16:0* | *16:1* | *17:0* | *18:0* | *18:1* | *19:0* |
|---|---|---|---|---|---|---|---|---|
| CIP 7135 | 2.58 | 0.13 | 37.43 | 4.20 | 0.60 | 1.15 | 33.50 | 20.37 |
| NCIB 4617 | 2.74 | 0.15 | 36.60 | 4.15 | 0.38 | 3.08 | 33.32 | 16.38 |

**Table 3** Sensitivity of *Lactobacillus brevis* strains to antibiotics

| *Antibiotic* | *Concentration* |
|---|---|
| Ampicillin | 10 μg |
| Bacitracin | 10 U |
| Cephaloridin | 30 μg |
| Chloramphenicol | 30 μg |
| Colistin | 10 μg[a] |
| Erythromycin | 15 μg |
| Kanamycin | 30 μg[a] |
| Methicillin | 5 μg[a] |
| Neomycin | 30 μg |
| Novobiocin | 5 μg |
| Penicillin | 10 U |
| Polymyxin B | 300 U[a] |
| Rifampicin | 5 μg |
| Streptomycin | 10 μg[a] |
| Tetracycline | 30 μg |

[a]Some strains are resistant.

Susceptibility of *L. brevis* to some antibiotics is presented in **Table 3**.

## Methods of Detection and Enumeration of *Lactobacillus brevis* in Foods

Many media have been described over the years for the isolation of lactobacilli from various foods. Semi-selective de Man, Rogosa and Sharp (MRS), all purpose Tween 80 (APT) and modified Homohiochii media (mHom) (**Table 4**) have been shown to be suitable as general culture media for isolating lactobacilli and other lactic acid bacteria.

Rogosa agar (RA) is commonly used when a selective medium is necessary for the detection of fastidious lactobacilli such as *L. brevis*. This medium, however, allows the growth of some pediococci, leuconostocs and yeasts (cycloheximide, 10 mg $l^{-1}$ can be added to inhibit yeasts). The use of RA is recommended for isolation from a wide variety of foods including milk and fermented milks, meat products, fermented vegetables, and salad dressings.

In some cases, it is difficult to detect *L. brevis*, as well as other microorganisms in foods. They are often present in low numbers, sublethally damaged due to the environmental conditions or may be adapted to specialized environments (fruit juices, wine, beer, etc.) and become very reluctant to multiply in other environments such as highly nutritious laboratory media. The addition of the natural substrate is often necessary to supply any unknown but essential growth factors.

**Table 4** Composition of some general media used for isolation of *Lactobacillus brevis* and other lactobacilli

| | *Medium* | | |
|---|---|---|---|
| *Component* | *MRS* | *APT* | *mHom*[a] |
| Peptone (g) | 10.0 | – | – |
| Tryptone (g) | – | 10.0 | 10.0 |
| Meat extract (g) | 10.0 | – | 2.0 |
| Yeast extract (g) | 5.0 | 5.0 | 7.0 |
| Glucose (g) | 20.0 | 10.0 | 5.0 |
| Fructose (g) | – | – | 5.0 |
| Maltose (g) | – | – | 2.0 |
| Na acetate.$3H_2O$ (g) | 5.0 | – | 5.0 |
| Na citrate (g) | – | 5.0 | – |
| Na gluconate (g) | – | – | 2.0 |
| $NH_4$ citrate (g) | 2.0 | – | 2.0 |
| $K_2HPO_4$ (g) | 2.0 | 5.0 | – |
| $MgSO_4.7H_2O$ (g) | 0.2 | 0.8 | 0.2 |
| $MnSO_4.4H_2O$ (g) | 0.05 | – | 0.05 |
| $MnCl_2.4H_2O$ (g) | – | 0.14 | – |
| $FeSO_4.7H_2O$ (g) | – | 0.04 | 0.01 |
| NaCl (g) | – | 5.0 | – |
| Mevalonic acid lactone (g) | – | – | 0.03 |
| Tween 80 (ml) | 1.0 | 1.0 | 1.0 |
| Cysteine HCl (g) | – | – | 0.5 |
| Agar (g) | 15.0 | 15.0 | 15.0 |
| Water (l) | 1.0 | 1.0 | 1.0 |
| pH | 6.2–6.5 | 6.7–7.0 | 5.4 |

[a]After sterilization, add 40 ml ethanol per litre.

**Table 5** Composition of orange serum agar for isolation and enumeration of spoilage organisms of citrus products

| *Component* | *Orange serum agar* |
|---|---|
| Tryptone (g) | 10.0 |
| Yeast extract (g) | 3.0 |
| Glucose (g) | 4.0 |
| $K_2HPO_4$ (g) | 3.0 |
| Orange extract (g) | 5.0 |
| Agar (g) | 17.0 |
| Water (l) | 1.0 |
| pH | 5.5 |

Media prepared with orange juice have been used for the control of the processing of citrus products. An orange serum agar (**Table 5**) has been developed for the isolation of microorganisms responsible for spoilage of citrus products. Due to their strongly stimulatory effects, tomato juice and yeast extract are normally included in media for the isolation of lactobacilli from wine. Addition of ethanol to all media is also recommended.

Many media have been used for isolation of beer

**Table 6** Composition of standard media used for enumeration of lactobacilli

| *Component* | *Raka-Ray agar*[a] | *Sucrose agar*[b] |
|---|---|---|
| Casein peptone (g) | – | 10.0 |
| Yeast extract (g) | 5.0 | 5.0 |
| Liver concentrate (g) | 1.0 | – |
| Tryptone (g) | 20.0 | – |
| Tween 80 | 10.0 ml | 0.1 g |
| Glucose (g) | 5.0 | – |
| Fructose (g) | 5.0 | – |
| Maltose (g) | 10.0 | – |
| Sucrose (g) | – | 50.0 |
| $NH_4$ citrate (g) | 2.0 | – |
| Potassium aspartate (g) | 2.5 | – |
| Potassium glutamate (g) | 2.5 | – |
| Potassium phosphate (g) | 2.0 | – |
| NaCl (g) | – | 5.0 |
| $MnSO_4.4H_2O$ (g) | 0.7 | 0.5 |
| $MgSO_4.7H_2O$ (g) | 2.0 | 0.5 |
| $CaCO_3$ (g) | – | 3.0 |
| *N*-Acetylglucosamine (g) | 0.5 | – |
| Betaine HCl (g) | 2.0 | – |
| Bromocresol green (mg) | – | 20.0 |
| Cycloheximide (mg) | 7.0 | – |
| Agar (g) | 17.0 | 20.0 |
| Distilled water (l) | 1.0 | 1.0 |
| pH | 5.4 | 6.2 |

[a]After autoclaving, just before pouring the plates add 3.0 g 2-phenyl ethanol.
[b]After autoclaving, just before pouring the plates add filter sterilized cycloheximide (final concentration 10.0 mg $ml^{-1}$) and 3.0 g 2-phenyl ethanol.

**Table 7** Composition of some media developed for enumeration of lactobacilli in beer

| | *Medium* | | |
|---|---|---|---|
| *Component* | *KOT* | *M NBB* | *UBA* |
| Casein peptone (g) | – | 5.0 | – |
| Peptonized milk (g) | – | – | 15.0 |
| Yeast extract (g) | 2.5 | 5.0 | 6.1 |
| Liver concentrate (g) | 1.0 | – | – |
| Trypticase peptone (g) | 5.0 | – | – |
| Malt extract (g) | 2.5 | – | – |
| Meat extract (g) | – | 2.0 | – |
| Glucose (g) | 5.0 | 15.0 | 16.1 |
| Maltose (g) | 5.0 | 15.0 | – |
| Tween 80 (ml) | 1.0 | 0.5 | – |
| Ferrous sulphate (mg) | – | – | 6.0 |
| Potassium acetate (g) | – | 6.0 | – |
| $K_2HPO_4$ (g) | 0.5 | 2.0 | 0.3 |
| $KH_2PO_4$ (g) | – | – | 0.3 |
| NaCl (mg) | – | – | 6.0 |
| $MnSO_4.4H_2O$ (mg) | 25.0 | – | 6.0 |
| $MgSO_4.7H_2O$ (g) | 0.12 | – | 0.12 |
| $NaN_3$ (mg) | 50.0 | – | – |
| Cysteine HCl (g) | 0.5 | 0.2 | – |
| Chlorophenol red (mg) | – | 70.0 | – |
| L-Malic acid (g) | 0.5 | 0.5 | – |
| Tomato supplement (g) | – | – | 12.2 |
| Cytidine (g) | 0.2 | – | – |
| Thymidine (g) | 0.2 | – | – |
| Cycloheximide (g) | 0.1 | – | – |
| Agar (g) | 20.0 | 15.0 | 12.0 |
| Beer (l) | 0.8 | 0.5 | 0.25 |
| Distilled water (l) | 1.0 | 0.5 | 0.75 |
| pH | 6.3 | 5.8 | 6.1 |

spoiling lactobacilli. Some of these media e.g. MRS medium (Table 4), MRS medium supplemented with maltose, Raka-Ray medium (**Table 6**) and sucrose medium (Table 6), are standard media for the detection of these organisms. Other media have been developed especially for the brewing industry and normally include wort or beer in their formulation (composition of some of these media is presented in **Table 7**), e.g. Nachweismedium fur bieerchadliche bacterien (NBB) and modified NBB medium (m NBB), Universal Beer Agar (UBA), VLB-S7-Agar, KOT medium, etc. A double-concentrated MRS medium adjusted with beer to normal concentration before autoclaving is also often used. Avoparcin (20 μg $ml^{-1}$) and vancomycin (30 μg $ml^{-1}$) are added to media used for isolation of beer spoilage organisms as these antibiotics inhibit the growth of most Gram-positive bacteria and have no effect on growth of heterofermentative lactobacilli, *L. salivarius* and bacteria of the genera *Leuconostoc* and *Pediococcus* which constitute the main beer spoilage lactic acid bacteria.

Although several comparisons between different media have been done the optimal medium for detection of lactobacilli in beer has not yet been identified. The use of Raka-Ray agar is recommended by the American Society of Brewing Chemists and the European Brewing Convention.

Plating methods take a long time (incubation time is normally 5–7 days at 28–30°C) to detect beer spoilage organisms and have generally a poor selectivity, so that more rapid alternative methods have been developed, e.g. bioluminescence techniques, direct epifluorescence filter techniques, immunoassays, conductimetric analysis, flow cytometry, polymerase chain reaction (PCR), DNA hybridization techniques, etc. As *L. brevis* is a common brewery contaminant that rapidly proliferates in beer, it is considered to be a suitable indicator for monitoring spoilage but most of the existing rapid methods are not efficient enough for its early detection or include the use of advanced, expensive equipment and reagents.

Bioluminescence methods, based on the measurement of light produced when ATP reacts with the firefly luciferin/luciferase enzyme system, can detect about 100 bacteria cells per millilitre. With this technique, however, detected microorganisms are not identified and it is difficult to determine numbers in beer which contains both yeast and bacteria. It is also

difficult to use bioluminescence as a routine analytical tool in breweries as the reagents are rather unstable. The basis of the direct epifluorescence filter techniques is the concentration of the cells on a membrane filter and staining them with acridine orange, a fluorescent dye, which binds to the nucleic acids. Viable cells fluoresce orange, non-viable cells fluoresce green. This technique has been used with success for milk but with heat-treated beverages differentiation between viable and non-viable cells is unreliable. Conductance measurements for the rapid detection of lactobacilli in beer has been investigated and has shown promise. Samples containing less than about 50 cfu $ml^{-1}$ were not detected within 50 h but higher levels were detected in 30 h or less. At present, however, these methods only indicate the presence or absence of contaminants and cannot be used when actual counts are required.

PCR assays involving amplification of DNA and RNA fragments were developed for the rapid detection of *L. brevis*. PCR assays are generally highly specific and sensitive, but the procedure is complicated and not suitable for use in the brewery as it involves time-consuming and delicate steps such as DNA extraction, PCR amplification, and electrophoresis. Additionally, the presence of PCR inhibitors in beer decreases the sensitivity of PCR assays which seem to be limited to laboratory use at present.

Recently, a new method has been developed in which imaging of single cells and microcolonies without a microscope by an ultrasensitive chemiluminescence enzyme immunoassay with a photon-counting television camera allows the rapid detection and quantification of *L. brevis* contaminants in beer and pitching yeast, i.e. the MicroStar Rapid Micro Detection System. It is claimed that optimization of membrane filtration, bioluminescent chemistry and advanced image analysis, enables the user to rapidly (within minutes or hours rather than days) enumerate 0–200 cfu per sample in variable sample volumes. This method compares well with PCR in terms of sensitivity but it is less labour-intensive and more rapid.

## Culture Maintenance and Conservation

As for most other species of lactobacilli, *L. brevis* can be cultured in MRS broth or yeast glucose chalk litmus milk medium, kept at 4°C, and periodically transferred to fresh medium or maintained for periods no longer than 1–2 months in MRS or tomato juice agar stabs. Addition of glycerol to the cultures (1 : 1) allows storage at −20°C for at last one year without significant loss of viability and reduces the risks of contamination, selection of mutants, loss of culture and transposition of strain numbers or designations associated with serial transfer techniques. Freezing in liquid nitrogen and freeze-drying are the recommended methods for long-term preservation. If these facilities are not available, cryogenic storage of the cells (with added glycerol) at −70°C on glass beads is also a good method.

## Importance in the Food Industry

### Fermented Products

Fermentation is considered to be one of the most economical methods of producing and preserving foods for human consumption. It is extensively used for these purposes in the underdeveloped world where the low levels of disposable income and limited infrastructure available in the food-processing industry greatly restrict the use of more advanced technologies.

*L. brevis* is involved in the production of a wide variety of fermented products (**Table 8**), reflecting the different diets and needs in various parts of the world. Some of these fermented foods have developed from natural fermentations into the selection and use of specific starter strains; however, even in Europe, several industrial lactic acid food fermentations are still 'spontaneous' processes.

In contrast to most vegetable fermentations, which are still produced on a small scale, sauerkraut, pickles and olives are fermented vegetables of significant commercial importance in the western world. Sauerkraut is made from salted shredded cabbage. Fermentation starts with *Leuconostoc mesenteroides*, present in high numbers in fresh cabbage, producing lactic acid, acetic acid and $CO_2$. Then *L. brevis* grows, producing more acid, and finally *L. plantarum* lowers the pH to below 4.0. The early dominance of heterofermenters

**Table 8** Application of *Lactobacillus brevis* in fermented foods

| *Product* | *Raw material* | *Area* |
|---|---|---|
| Burong mustala | Mustard | Philippines |
| Busaa | Maize, finger millet, sorghum | Kenya |
| Cheese | Milk | Worldwide |
| Fufu | Cassava tubers | Nigeria |
| Kefir | Milk | Caucasus |
| Kimchi | Korean cabbage, Korean radish root | Korea |
| Kishk | Milk, wheat | Egypt, Iraq |
| Laban zeer | Sour milk | Egypt |
| Mesu | Bamboo shoot | India |
| Nham | Pork, rice | Thailand |
| Olives | Green olives | Worldwide |
| Pickles | Vegetables, cucumbers | Worldwide |
| Pulque | Agave juice | Mexico |
| Sauerkraut | White cabbage | Worldwide |
| Sausages | Pork, beef | Worldwide |
| Sourdoughs | Wheat, rye | Worldwide |

in the fermentation is important in the inhibition of undesirable organisms ensuring the stability and consistency of the natural fermentation process. Although they produce less total acidity, acetic acid, with a higher p*K*a, is a more potent antimicrobial than lactic acid. Various studies have been performed to develop starter cultures to sauerkraut fermentation but industrial production is still based on natural fermentation processes.

For the production of pickled cucumbers, whole vegetables are washed and covered with brine. Aerobic microorganisms develop first and create favourable conditions for the growth of lactic acid bacteria (LAB) which are responsible for the main fermentation process. The succession of LAB in cucumber fermentation is similar to that of sauerkraut: the heterofermentative LAB, initially *Leuconostoc* spp. followed by *L. brevis*, are soon overgrown by homofermentative species such as *L. plantarum* and *Pediococcus pentosaceus*.

A sourdough for leavening bread doughs, is one of the oldest biotechnological processes in food production. Although nowadays breads from wheat may be leavened with yeasts exclusively, sourdoughs containing *Lactobacillus* (*L. brevis*, *L. delbrueckii*, *L. fermentum*, *L. plantarum*, *L. sanfrancisco* and others) are still used mainly in the production of rye and rye–mixed grain breads, cake leavened baked products (e.g. Panettone) and wheat bread. The use of 'spontaneous' fermentation to produce a sourdough results in small deviations between fermentations because the composition of the microflora is not critically controlled. It is known that heterofermentative strains of lactic acid bacteria are needed to obtain the sensory properties characteristically associated with sourdough breads. Although the application of starter cultures for the production of fermented foods of plant origin has still not been very successful in practice, some lactic acid bacteria strains including *L. brevis* are now being industrially produced in highly concentrated freeze-dried form. It is convenient and quick to use these cultures to make sourdough bread.

Nitrite reduction is a rare property of lactic acid bacteria. Two types of nitrite reductases are known, those depending on the presence of haematin (ammonia is produced from nitrite reduction) and haem-independent enzymes (NO and $N_2O$ are produced from nitrite reduction). *L. brevis* possesses haem-independent nitrite reductases. This is an important characteristic for technological or toxicological purposes with regard to potential applications as starter cultures in food fermentations. The production of NO is desirable in meat technology since this intermediate is required in the reddening reaction. It is usually produced from nitrite in chemical reactions and provides the substrate for the formation of nitrosomyoglobin. The production of $N_2O$ might be an advantage over ammonia since it is more effective and it requires less reduction equivalents to remove nitrite from the substrate.

Kefir grains, which are necessary to inoculate milk to produce Kefir (Table 8), are conglomerates of lactic acid bacteria and yeasts held together by a polysaccharide gum. This polysaccharide, kefiran, is produced by the predominating bacterial species, including *L. brevis*.

The secondary flora of many hard and semihard cheeses, such as Cheddar, Gouda and Edam, is dominated by mesophilic lactobacilli such as *L. brevis*. Their exact role in the cheese is not fully understood, but it is considered that they have an important function in flavour development. The substrates used as energy sources within the ripening cheese are not well known. Since only residual amounts of lactose are present, insufficient to support significant growth of lactobacilli, other sources of metabolites/nutrients must be considered (e.g. galactose, citrate, lactate, starter cell autolysate material, free amino acids, peptides and glycerol from lipolysis).

The importance of silage in the diet of ruminants is well established. The increasing practice of conserving fodder as silage, rather than hay, for feeding cattle in winter has been one of the most important developments in farming in the past several decades. Silage is made from various raw materials, of which grass, hay and maize play the major role. *L. brevis* and some other lactobacilli are very important in the acidification of silage made from green forage. Silage acidification is normally initiated by homofermentative lactic acid bacteria. As fermentation proceeds, *L. brevis* and other heterofermentatives, become dominant.

### Food Spoilage

*L. brevis* in some circumstances can be a cause of spoilage of various food products. The organism is commonly isolated from grapes and wines worldwide. It is possible that some strains or species could contribute desirable characteristics to wine quality, although excessive growth could be undesirable. When present in trace amounts, diacetyl enhances wine flavour. However, excessive production of diacetyl from citric acid by lactobacilli causes spoilage. *L. brevis* produces mannitol from glucose. The production of ethanol and glycerol decreases as fructose is reduced to mannitol. Furthermore, the excess production of mannitol may result in mannite spoilage of wine. Mannite spoilage is accompanied by formation of excess acetic acid. The decomposition of tartaric acid is normally associated with severe spoil-

**Table 9** Some characteristics of *Lactobacillus brevis* important for beer spoilage

| | |
|---|---|
| Hop tolerance[a] | 25–35 |
| Maltose | + |
| $O_2 < 0.4$ (mg $l^{-1}$) | + |
| pH | < 4.2 |
| Alcohol tolerance | > 14% |
| Minimum temperature | 2–4°C |

+, Growth.
[a]EBC bitterness unit.

age of wine. Tartarate decomposition by *L. brevis* results in the formation of $CO_2$, lactate, acetate and succinate.

Sorbate in wines, generally around 200 p.p.m., although being inhibitory to most yeasts and some lactic acid bacteria, is not inhibitory for *L. brevis* which shows almost no inhibition by sorbate levels up to 1000 p.p.m. Addition of $SO_2$ to crushed grapes (minimum 30 ml $l^{-1}$) has proved useful to delay the growth of *L. brevis*.

*L. brevis* is potentially one of the most undesirable beer spoilage microorganisms because of its microaerophilic nature, its resistance to hop-derived compounds, such as isohumulone, to ethanol and to low pH (**Table 9**). Beer spoilage by lactobacilli is characterized by 'silky' turbidity accompanied by acid, 'dirty' (acetoin) or 'buttery' (diacetyl) off-flavours.

One of the major bacterial spoilage agents in citrus juices is *L. brevis*. This organism can multiply at a pH of < 3.5 and at a temperature of 10°C and is responsible for the production of diacetyl which imparts an undesirable 'buttery' flavour to juice, and fermented off-flavours due to ethanol, carbon dioxide and higher-molecular-weight alcohols.

Heterofermentative isolates from cider are usually *L. brevis*. They metabolize fructose actively to produce acetate which is detrimental to flavour.

*L. brevis* has also been responsible for gas production in salad dressings, vigorous fermentation in canned tomato resulting in can swelling and acid odour in canned tomato ketchup, Worcestershire sauce and similar products, milk stringiness produced by the growth of cord like strains, production of carbon dioxide in marinated herring, and coloured spots in cheese as a consequence of growth of orange-pigmented strains. If present in excessive numbers (> $10^8$ cfu $ml^{-1}$), *L. brevis* can be responsible for certain cheese defects: undesirable gas pockets and blowing of packaged cheeses due to excessive production of $CO_2$, formation of biogenic amines, 'green spot' development, excessive build up of calcium lactate crystals, unclean flavours, and acidic texture and flavour.

## Importance for the Consumer

As previously noted, *L. brevis* occurs naturally in different food materials or can be deliberately introduced in order to produce different fermented foods (Table 8). In addition to the preservation effect, fermentation is a means of improving sensory quality and acceptability of many raw materials, improving the nutritional value and providing the consumer with a wide variety of flavours, aromas and textures to enrich the human diet. Additionally, preparation of lactic acid-fermented products has low, if any, energy requirements and can be consumed without (or with little) cooking (e.g. pickled vegetables, fermented cabbages, olives). Energy saving is very important in countries where housewives spend many hours collecting leaves, twigs, wood and dried dung with which to cook every day.

### Natural Preservation

The ability of lactic acid bacteria to inhibit growth and survival of spoilage microflora and pathogens has been used as a means of improving safety and keeping quality of foods. Whereas in the Western world foods are preserved by refrigeration, freezing, canning or modified atmosphere packaging, in developing countries these techniques are prohibitively expensive and fermentation and drying are the methods available. The contribution of lactic acid fermentations to food safety is very important in developing countries.

The use of 'natural' methods of preservation has increased during recent years when the nutrient content of processed foods became a concern among consumers. Fermentation is a useful natural preservation system that meets consumer concerns.

The decrease in pH during fermentation creates an environment that is unfavourable to pathogens and spoilage organisms. Additionally, *L. brevis* produces significant quantities of acetic acid which is a more effective antimicrobial agent than lactic acid. Although acidity is the most important antimicrobial factor, other inhibitory agents produced by *L. brevis* should not be ignored, e.g. bacteriocins, $CO_2$, hydrogen peroxide, ethanol and diacetyl.

Bacteriocins are antimicrobial compounds, containing a biologically active protein/peptide moiety, produced by bacteria which are inhibitory to a limited range of organisms, normally very closely related bacteria. Various *L. brevis* strains produce bacteriocins (**Table 10**).

The antimicrobial activity of $CO_2$, hydrogen peroxide, ethanol and diacetyl is well established. The individual contribution of each of these agents, however, is relatively minor, particularly compared with the acid production that occurs at the same time

**Table 10** Bacteriocins produced by *Lactobacillus brevis* strains

| *Bacteriocin producer* | *Bacteriocin* | *Activity against* |
|---|---|---|
| Isolated from kimchi | Unnamed | *Enterococcoccus faecalis, E. faecium, L. brevis, L. sake, Leuc. mesenteroides, P. pentosaceus* |
| *L. brevis* B37 (isolated from plant and fermenting material) | Brevicin 37 | *L. brevis, Leuc. oenos, Nocardia corallina, P. damnosus* |
| *L. brevis* SB27 (isolated from sausages) | Brevicin 27 | *L. brevis, L. buchneri, L. plantarum, P. pentosaceus*, some *Bacillus* spp. |
| *L. brevis* VB286 (isolated from vacuum packaged meat) | Brevicin 286 | *E. faecalis, E. faecium, L. curvatus, Listeria* spp. |

(reduced pH and presence of undissociated organic acids).

### Nutritional Value and Health Considerations

**Improvement in Nutritional Value and Health Benefits** Increased nutritional quality of fermented foods has been attributed to improvement in the nutrient density, increase in the amount and bioavailability of nutrients, detoxification of food raw materials, improvement of functional properties, and improvement in digestibility. The role of individual microorganisms in the increased nutritional value of these products is sometimes unclear since investigations on the microorganisms involved in most of the fermentation processes appear to terminate at the isolation and identification stages.

Fermentations which involve yeasts tend to be enriched in the B vitamins. Pulque (Table 8), produced by the fermentation of juices of agave, continues to be an important source of nutrition of peasants and other low-income people in the poorest semi-arid areas of Mexico (agave is the only plant that can grow on the very poor soil under the extremely low water availability). Pulque is rich in thiamin, riboflavin, niacin, pantothenic acid, *p*-aminobenzoic acid, pyridoxine and biotin. Additionally, ethanol present in pulque is an important source of calories.

Microbial activity during the production of fufu (Table 8) softens cassava root tissues allowing linamarase to breakdown linamarin, a cyanogenic glycoside responsible for severe intoxications following the consumption of raw cassava. *L. brevis* isolated from fermented cassava products possessed considerable linamarase activity but did not possess tissue-degrading enzymes.

In legumes, carbohydrates are often present as oligosaccharides, such as raffinose, stachyose and verbascose, which are not readily digestible, and can cause flatus, diarrhoea and indigestion when broken down by bacteria in the large intestine. These oligosaccharides possess α-D-galactosidic bonds which are hydrolysed by α-galactosidases. α-Galactosidase production is a constitutive property of *L. brevis*.

Surpluses of vegetables can be safely preserved by farmers using lactic acid fermentation (e.g. pickled vegetables). This improves the supply and availability of vegetable foods throughout the year and improves the nutrition of the population. For example, kimchi (Table 8) is an important source of vitamins and minerals in Korea during the wintertime when fresh vegetables are not available.

Various *L. brevis* strains produce γ-aminobutyric acid, reported as having antihypertensive and diuretic effects.

**D-Lactate** D-Lactate is a non-physiological isomer in mammals, which is poorly metabolized, and accumulates in the blood, especially if there is thiamin deficiency, causing acidosis (disturbance of the acid–alkali balance in the blood) and mineral mismanagement. The FAO/WHO Joint Committee on Food Additives reviewed the toxicological evidence available and concluded that there was evidence that babies in their first 3 months of life have difficulties in utilizing small amounts of DL or D-lactate and that neither should be used for infant foods.

**Biogenic Amines** The formation of biogenic amines of toxicological significance occurs in foods and *L. brevis* has been identified as the causative agent (e.g. tyramine in Gouda cheese). γ-Aminobutyric acid, cadaverine and histamine are formed during spoilage of food products by lactobacilli including *L. brevis*.

**Pathogenicity** *L. brevis* is generally considered to be non-pathogenic. However, it is associated with lung infections, complicated by lung cancer, indicating an opportunistic behaviour.

*See also:* **ATP Bioluminescence**: Application in Beverage Microbiology. **Bacteria**: Classification of the Bacteria – Phylogenetic Approach. **Bacteriocins**: Potential in Food Preservation. **Biochemical and Modern Identification Techniques**: Microfloras of Fermented Foods. **Bread**: Sourdough Bread. **Cheese**: Microbiology of Cheese-making and Maturation; Role of Specific Groups of Bacteria. **Direct Epifluorescent Filter Techniques (DEFT). Electrical Techniques**: Food Spoilage Flora and Total Viable Count (TVC); Lactics and other Bacteria.

**Fermented Foods**: Origins and Applications; Fermented Vegetable Products; Fermented Meat Products; Fermentations of the Far East. **Fermented Milks**: Range of Products; Yoghurt; Products from Northern Europe. ***Lactobacillus***: Introduction. **Starter Cultures**: Uses in the Food Industry; Importance of Selected Genera. **Wines**: Microbiology of Wine-making. **Preservatives**: Traditional Preservatives – Organic acids.

## Further Reading

Coventry MJ, Wan J, Gordon JB, Mawson RF and Hickey MW (1996) Production of brevicin 286 by *Lactobacillus brevis* VB286 and partial characterization. *Journal of Applied Bacteriology* 80: 91–98.

Gasser F (1994) Safety of lactic acid bacteria and their occurrence in human clinical infections. *Bulletin Institute Pasteur* 92: 45–67.

Hammes WP, Weiss N and Holzapfel W (1992) The genera *Lactobacillus* and *Carnobacterium*. In: Balows A, Truper HG, Dworkin M, Harder W and Schleifer KH (eds) *The Prokaryotes*, 2nd edn. Pp. 1535–1594. New York: Springer-Verlag.

Hammes WP and Vogel RF (1995) The genus *Lactobacillus*. In: Wood BJB and Holzapfel WH (eds) *The Lactic Acid Bacteria. The Genera of Lactic Acid Bacteria*, pp. 19–54. London: Blackie Academic.

Jespersen L and Jakobsen M (1996) Specific spoilage organisms in breweries and laboratory media for their detection. *International Journal of Food Microbiology* 33: 139–155.

Kandler O and Weiss N (1986) Regular, nonsporing Gram-positive rods. In: Sneath PHA, Mair NS, Sharp ME and Holt JG (eds) *Bergey's Manual of Determinative Bacteriology*. Vol. 2, p. 1209. Baltimore: William and Wilkins.

Kyriades AL and Thurston PA (1989) Conductance techniques for the detection of contaminants in beer. In: Stannard CJ, Petitt SB and Skinner FA (eds) *Rapid Microbiological Methods for Foods, Beverages and Pharmaceuticals*. Pp. 101–117. London: Blackwell Scientific Publications.

Saier Jr. MH, Ye JJ, Klinke S and Nino E (1996) Identification of an anaerobically induced phosphoenolpyruvate-dependent fructose-specific phosphotransferase system and evidence for the Embden–Meyerhof glycolytic pathway in the heterofermentative bacterium *Lactobacillus brevis*. *Journal of Bacteriology* 178: 314–316.

Steinkraus KH, Cullen RE and Gavitt BK (1983) *Handbook of Indigenous Fermented Foods*. New York: Marcel Dekker.

Vandamme P, Bot B, Gillis M et al (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiological Reviews* 60: 407–438.

Wood BJB (1998) *Microbiology of Fermented Foods*, 2nd edn. London: Blackie Academic and Professional.

Yasui T and Yoda K (1997) Imaging of *Lactobacillus brevis* single cells and microcolonies without a microscope by an ultrasensitive chemiluminescent enzyme immunoassay with a photon-counting television camera. *Applied Environmental Microbiology* 63: 4528–4533.