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International Journal of Food Microbiology 36 (1997) 1–29

International Journal of Food Microbiology

Review article

# Lactic acid bacteria of foods and their current taxonomy

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Received 8 May 1996; received in revised form 25 November 1996; accepted 25 November 1996

**Abstract**

Application of molecular genetic techniques to determine the relatedness of food-associated lactic acid bacteria has resulted in significant changes in their taxonomic classification. During the 1980s the genus *Streptococcus* was separated into the three genera *Enterococcus*, *Lactococcus* and *Streptococcus*. The lactic acid bacteria associated with foods now include species of the genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*[1]. The genus *Lactobacillus* remains heterogeneous with over 60 species (ymol% G + C content ranging from 33 to 55), of which about one-third are strictly heterofermentative. However, many changes have been made and reorganization of the genus along lines that do not follow previous morphological or phenotypic differentiation from *Leuconostoc* and *Pediococcus* is being studied. Phylogenetically belonging to the Actinomyces branch of the bacteria, *Lactobacillus bifidus* has been moved to the genus *Bifidobacterium* also on account of its greater than 50 mol% G + C content. It is therefore no longer considered one of the lactic acid bacteria senso strictu, which form part of the Clostridium branch of the bacteria. The new genus *Weissella* has been established to include one member of the genus *Leuconostoc* (*Leuc. paramesenteroides*) and heterofermentative lactobacilli with unusual interpeptide bridges in the peptidoglycan. Contrary to the clear-cut division of the streptococci, morphological and physiological features of *Weissella* do not directly support this grouping which now incorporates species that produce D(−)- as well as DL-lactate. The new genus *Carnobacterium* is morphologically similar to the lactobacilli, but it shares some physiological similarities (e.g. growth at pH 9.5) and a common phylogenetic branch with the genus *Enterococcus*. The review includes information on the taxonomic changes and the relationship of the bacteria to food fermentation and spoilage. © 1997 Elsevier Science B.V.

*Keywords:* *Carnobacterium*; *Enterococcus*; *Lactobacillus*; *Lactococcus*; *Leuconostoc*; *Oenococcus*; *Pediococcus*; *Streptococcus*; Phylogeny; Phenotypic properties; Lactic acid isomers; Starter cultures; Protective cultures; Biopreservation; Bacteriocins; Spoilage

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[1] Unconventional abbreviations *Lb.*, *Lc.* and *Leuc.* are used in this text to avoid confusion between genera *Lactobacillus*, *Lactococcus* and *Leuconostoc*, respectively.


*PII* S0168-1605(96)01233-0

Table 1
Orla-Jensen (1919) key to differentiation of the lactic acid bacteria and current taxonomic classification

| Genus[a] | Shape | Catalase | Nitrite reduction | Fermentation | Current genera |
|---|---|---|---|---|---|
| Betabacterium | Rod | – | — | Hetero- | *Lactobacillus*<br>*Weissella* |
| Thermobacterium | Rod | — | — | Homo- | *Lactobacillus* |
| Streptobacterium | Rod | – | — | Homo- | *Lactobacillus*<br>*Carnobacterium* |
| Streptococcus | Coccus | — | — | Homo- | *Streptococcus*<br>*Enterococcus*<br>*Lactococcus*<br>*Vagococcus* |
| Betacoccus | Coccus | — | – | Hetero- | *Leuconostoc*<br>*Oenococcus*<br>*Weissella* |
| Microbacterium | Rod | + | + | Homo- | *Brochothrix* |
| Tetracoccus | Coccus | +[b] | + | Homo- | *Pediococcus*<br>*Tetragenococcus* |

[a] According to Orla-Jensen (1919).
[b] In general pediococci are catalase negative but some strains produce a pseudocatalase that results in false positive reactions.

## 1. Introduction

The concept of the lactic acid bacteria (LAB) as a group of organisms developed at the beginning of the 1900s, preceded by pioneering scientific and technical developments during the latter part of the 19th century. The interactions of LAB in foods enjoyed early attention of scientists and resulted in the significant contribution by Pasteur on lactic acid fermentation in 1857, followed by the first isolation of a pure bacterial culture, *Bacterium lactis*, by Lister in 1873. Use of starter cultures for cheese and sour milk production was introduced almost simultaneously in 1890 by Weigmann in Kiel and Storch in Copenhagen. This opened the way for industrialisation of food fermentations. LAB are typically involved in a large number of spontaneous food fermentations but they are also closely associated with the human environment. Excavations in Switzerland established that sourdough bread was part of a typical diet over 5000 years ago (Währen, 1990) and the 'leavening' of (sourdough) bread was mentioned in the Bible (e.g. Matthew 13, 33). Reference to fermented dairy products (cheese, yoghurt, butter) is documented in archaic texts from Uruk/Warka (Iraq), dated around 3200 B.C. (Nissen et al., 1991). Beer brewed by the Babylonians and exported to Egypt around 3000 B.C. was most likely the product of both alcoholic and lactic fermentations. Present day sorghum, maize and millet beers in Africa possess similar features in which the lactic fermentation plays a key role in safety and acceptability of these products in tropical climates (Haggblade and Holzapfel, 1989). Several of the early 'empirical' biotechnological processes are still in use, but they are applied under well controlled conditions on an industrial scale, e.g. sauerkraut and cucumber fermentations. Some manufacturers still rely on traditional technologies for processing of cheese and fermented meat products without the use of starter cultures.

Early definitions of LAB as a group, based on the ability to ferment and coagulate milk, included the coliform bacteria with the lactics. The description of *Lactobacillus* organisms by Beijerinck in 1901 as gram-positive bacteria separated the coliforms from the LAB. The influence of selected lactobacilli in various food fermentations has been well established since then. According to Orla-Jensen (1919) the 'true lactic acid bacteria' form a natural group of gram-positive, nonmotile, non-sporeforming, rod- and coccus-shaped organ-

Table 2
Sherman's (1937) classification system for the streptococci

| | Sherman's group | | | |
|---|---|---|---|---|
| | Pyogenic | Viridans | Lactic | *Enterococcus* |
| Lancefield's group | A, B, C, G | –[a] | N | D(Q) |
| Haemolysis | $\beta$ | $\alpha$ | – | – |
| Growth | | | | |
| 10°C | – | – | + | + |
| 45°C | (+) | + | – | + |
| pH 9.6 | – | – | – | + |
| Survive 60°C for 30 min | – | + | Variable | + |
| Methylene blue (0.1%) | – | – | + | +(–) |

[a] Various groups or negative.

isms that ferment carbohydrates and higher alcohols to form chiefly lactic acid. He proposed the seven genera shown in Table 1. Interest in the lactobacilli in the human diet was great at the turn of the 20th Century when Elie Metchnikoff at the Pasteur Institute in Paris promoted their use in the diet for bacterioprophylaxis and bacteriotherapy (Bibel, 1988); but his theories of healthfulness and longevity through consumption of lactobacilli fell into disrepute because of unrealistic consumer expectations and insufficient scientific-based evidence. When dividing the catalase negative, rod-shaped LAB into three genera (Table 1) Orla-Jensen (1919) included Metchnikoff's strain from Bulgarian yoghurt as *Thermobacterium bulgaricum*. Claims that LAB are important in human and animal health are receiving renewed attention as health promoting (probiotic) agents, especially strains showing a stabilizing or favourable influence on the gastrointestinal tract (Fuller, 1989).

Orla-Jensen's (1919) monograph on the LAB emphasised the importance of the streptococci in milk and dairy products. The first systematic classification of the streptococci was proposed by Sherman (1937). Strict anaerobes and the pneumococci were excluded from the classification because of their extreme sensitivity to bile. The remaining facultatively anaerobic streptococci were divided into the four groups shown in Table 2. It is remarkable that these key physiological characteristics provided sufficient justification for a grouping that to a large extent was confirmed by Schleifer and Kilpper-Bälz (1984) and Schleifer and Kilpper-Bälz (1987) when suggesting the new genera *Lactococcus* and *Enterococcus* based on the molecular characteristics. Lancefield (1933) had earlier proposed the serological differentiation of the streptococci based on their so-called 'C substances'. Good correlation of Lancefield's groups A–E and N was observed with physiological and biochemical tests (Sherman, 1937). Heavy reliance on serological grouping of the streptococci developed; however, as the number of serological groups increased to 20, the correlation between serotype and biotype decreased.

The classical approach to bacterial taxonomy was based on morphological and physiological features. This was expanded to include the cell wall composition, cellular fatty acids, isoprenoid quinones and other characteristics of the cells. Molecular characteristics have become important taxonomic tools, such as the mol% G + C content of the DNA, electrophoretic properties of the gene products, DNA:DNA hybridisation studies and structures and sequence of ribosomal RNA (rRNA). This has resulted in dramatic changes in taxonomy of the LAB (Schleifer, 1987). The classification of LAB remains volatile and it forms the focus of intense taxonomic study with an increasing urgency for a polyphasic approach involving both phenotypic and phylogenetic characterisation of bacteria (Vandamme et al., 1996).

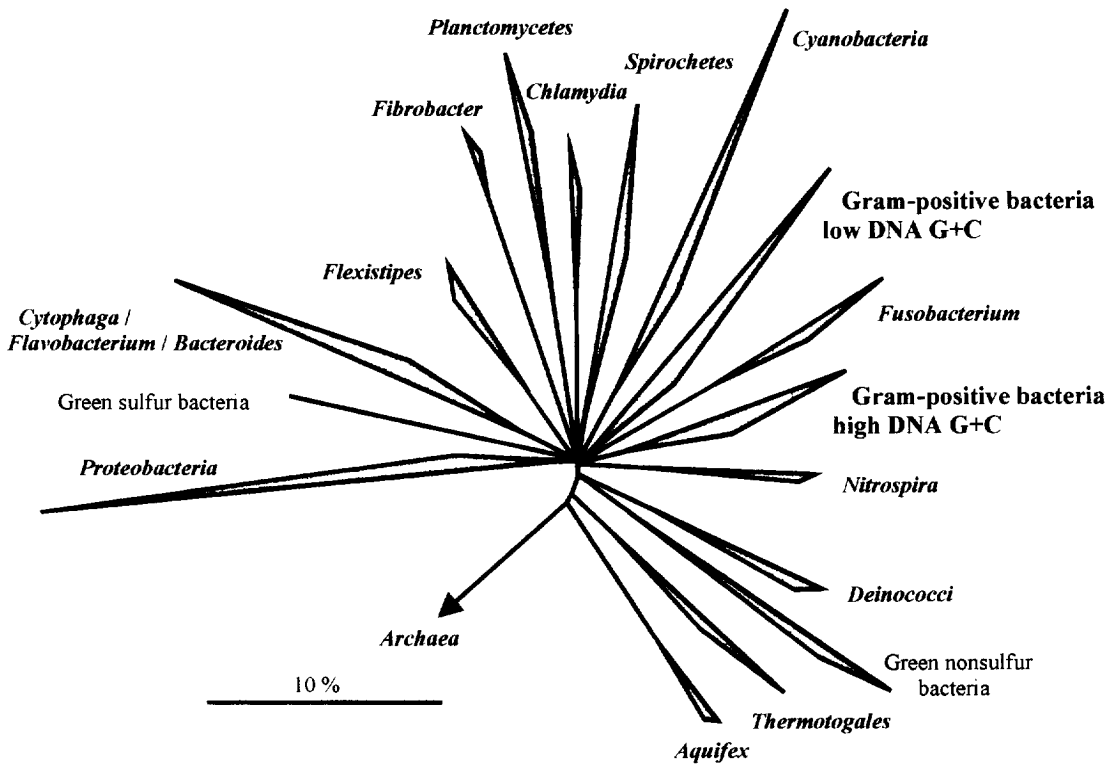


Fig. 1. Consensus tree showing major lines of descent among the bacteria, based upon comparative analysis of 16S rRNA sequences (modified according to Schleifer and Ludwig, 1995).

There have been reports and reviews of the involvement of LAB in human clinical infection, culminating in the reviews by Aguirre and Collins (1993) and Gasser (1994). They acknowledged the beneficial role of LAB in food preservation and probiotics, but they cited numerous cases in which LAB have been implicated in human disease. Aguirre and Collins (1993) focused attention on the importance of enterococci in clinical infection; however, reviews of lactobacilli (Sussman et al., 1986) and leuconostocs (Handwerger et al., 1990) in human infections concluded that some of these bacteria should be viewed as 'potential pathogens'. In the vast majority of these clinical cases, patients either had a history of underlying disease or should be considered as immunocompromised and may have been treated with antibiotics, in particular vancomycin. This lead Aguirre and Collins to conclude that these lactics fall into the category of opportunistic pathogens. Even this statement is speculative because the conditions for their behaviour as opportunistic pathogens, including distinguishing physiological features, have not been defined. Clearly, food scientists should be cognisant of the association of LAB with clinical infections but there is no evidence that fermented foods are a concern in the diet.

When food microbiologists referred to the LAB or the 'lactics' they did so generally with reference to certain species of the genera *Lactobacillus*, *Lactococcus* (*Streptococcus*), *Leuconostoc* and *Pediococcus*. Changes based on chemotaxonomic and phylogenetic studies have resulted in dramatic and on-going changes in their nomenclature. Phylogenetically the LAB belong to the clostridial branch of the gram-positive bacteria. They are catalase negative, nonsporeforming, cocci, coccobacilli or rods that have less than 55 mol% G + C content in their DNA. This distanced these 'traditional' lactics from the bifidobacteria which

have greater than 55 mol% G + C in the DNA and therefore belong to the 'Actinomyces' branch of bacteria (Fig. 1) (Schleifer and Ludwig, 1995). The LAB of importance in foods belong to the genera: *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Vandamme et al., 1996). The 16S rRNA data for the LAB suggest new groupings that cross established taxonomic lines. Not all of the new groupings have become established in bacterial taxonomy, but recent phylogenetic considerations indicate that the lactobacilli, leuconostocs and pediococci will be reclassified as three major groups (Collins et al., 1993b; Schleifer and Ludwig, 1995; Vandamme et al., 1996) the *Leuconostoc* group, the *Lb. delbrueckii* group and the *Lb. casei-Pediococcus* group (Table 11). The newly established genera *Carnobacterium* (atypical, acid-sensitive lactobacilli), *Tetragenococcus* (previously *Pediococcus halophilus*) and *Vagococcus* (previously motile streptococci) form a phylogenetic cluster with the genus *Enterococcus*. Some of these relationships are indicated in Fig. 2, adopted from Schleifer and Ludwig (1995). This review considers the current taxonomy of the LAB and closely related bacteria of importance in the microbial ecology of foods.

## 2. Streptococci

Streptococci were among the earliest bacteria to be recognised by microbiologists because of their involvement in a large number of human and animal diseases. The generic name *Streptococcus* was first used by Rosenbach (1884) to describe the chain-forming, coccus-shaped bacteria associated with wound infections. The genus *Streptococcus* was originally described based on morphological, serological, physiological and biochemical characteristics and it comprised a wide range of organisms including the highly pathogenic bacteria *S. pneumoniae*, *S. pyogenes* and *S. agalactiae*; the intestinal group D streptococci *S. faecalis* and *S. faecium*; and the economically important group N starter bacteria *S. cremoris* and *S. lactis*. Jones (1978) reviewed the composition and differentiation of the genus *Streptococcus* and proposed seven groups, including the strict anaerobes and pneumococci, based on artificial criteria of pathogenicity, habitat and oxygen tolerance, that "do not necessarily imply any close relationship between the streptococci included in any one group." The streptococci have complex nutritional requirements and thrive in environments with a good supply of carbohydrate and protein, including tissues and intestinal tracts of animals, milk, dairy products, vegetable material and other foods (Jones, 1978).

Based on a molecular approach, the genus *Streptococcus* has undergone major revisions. Studies by Kilpper-Bälz et al. (1982) on the 23S rRNA homology clusters of the serological groups N and D streptococci revealed three homology groups. The evolutionary tree of the *Clostridium* branch of the gram-positive bacteria, based on 16S rRNA sequence similarities (Stackebrandt and Teuber, 1988), indicated the appropriateness of separating the streptococci into three genetically distinct groups: *S. sensu stricto*, *Enterococcus* and *Lactococcus* (Schleifer and Kilpper-Bälz, 1984, 1987). The species remaining in the genus *Streptococcus* include the pathogenic and the oral (Sherman's Viridans group) streptococci.

*S. thermophilus* is an exception in this genus because it is an important starter organism for yogurt and cheese manufacture. The taxonomy of *S. thermophilus* has been controversial (Jones, 1978). It was placed in the Viridans group by Sherman (1937). It does not produce a Lancefield group antigen response. *S. thermophilus* grows at 45°C and up to 50°C, but not at 15°C and it is relatively heat resistant. Attempts by London and Kline (1973) to classify it with the group N streptococci could not be justified (Kilpper-Bälz et al., 1982). *S. thermophilus* was thus, retained in the genus *Streptococcus* and reclassified with the 'oral streptococci', in fact, it was considered a subspecies of *S. salivarius* (Farrow and Collins, 1984). However, further DNA hybridisation studies showed that it was clustered with the oral streptococci *S. salivarius* and *S. vestibularis* but that assignment of separate species status for *S. thermophilus* was justified (Schleifer et al., 1991). To-

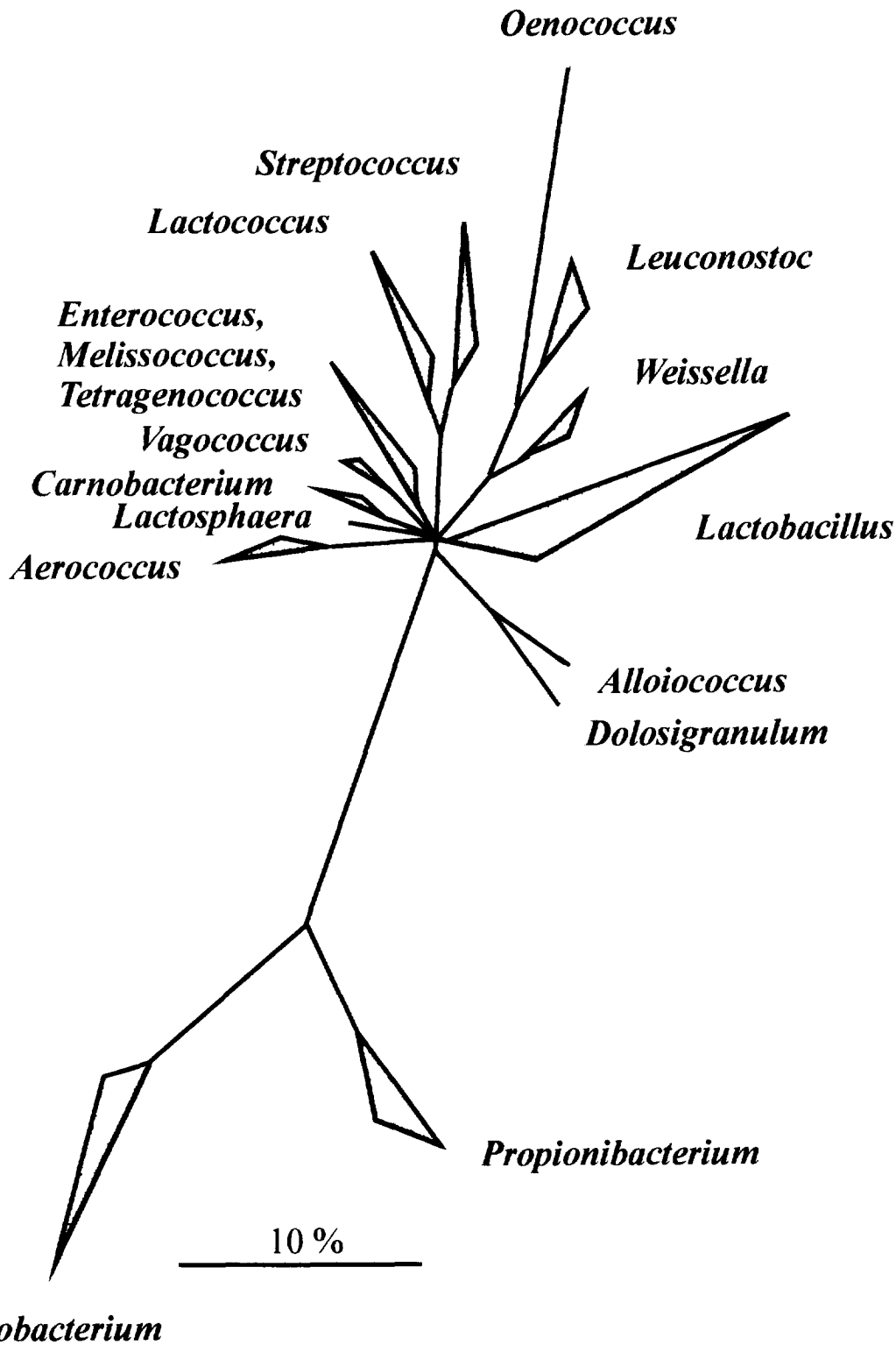


Fig. 2. Major phylogenetic groups of lactic acid bacteria and related gram-positive bacteria with low (upper part) and high (lower part) mol% G + C in the DNA (modified according to Schleifer and Ludwig, 1995).

gether with *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. lactis* and (or) *Lb. helveticus*, it is used in mixed strain starter cultures with an optimum incubation temperature above 40°C, such as in yogurt and related fermented milks, as well as in Swiss- and Italian-type cheeses. *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* have a special relationship of associative growth in which the *Streptococcus* strain produces formic acid that promotes the growth of the *Lactobacillus* which, on its turn, provides flavour compounds (acetaldehyde) and the proteolytic activity to keep the *Streptococcus* strain growing in milk. This is the only organism still classified as a *Streptococcus* that is used as a starter culture in foods.

## 3. Lactococci

Most but not all of the Lancefield group N lactic streptococci have been transferred to the genus *Lactococcus* (Schleifer et al., 1985). The ovoid shape of the lactococci can be difficult to interprete because the cells are sometimes elongated in the plane of chain formation (appearing as coccobacilli) resulting in the misclassification of some lactococci as lactobacilli. Based on the studies of Schleifer et al. (1985) there was the revolutionary reclassification of *Lb. xylosus* as *Lc. lactis* subsp. *lactis* and *Lb. hordniae* as *Lc. lactis* subsp. *hordniae*. The genus *Lactococcus* includes several uncommon species: *Lc. garvieae* associated with mastitis in cows, *Lc. piscium* from salmonid fish, *Lc. plantarum* from frozen peas and *Lc. raffinolactis* from raw milk (Schleifer et al., 1985; Williams et al., 1990). Not all strains that react with the group N antisera have been reclassified as lactococci. The motile group N streptococci are included in the genus *Vagococcus*.

The subspecies of *Lc. lactis* are of great economic importance and have been extensively studied for their biochemical and physiological characteristics, and their effect on foods (Teuber et al., 1991). The subspecies of *Lc. lactis* are the most important of the commercially used LAB. *Lc. lactis* is commonly isolated from plant material (Sandine et al., 1972), but the most recognised habitat for the lactococci is dairy products. They are nonmotile, coccus-shaped homofermentative bacteria that grow at 10°C but not at 45°C and produce L(+)-lactic acid from glucose. Through acquisition of plasmid DNA encoding the phosphoenolpyruvate–phosphotransferase system (PEP–PTS) some strains became well adapted to grow in milk because of their efficient uptake and fermentation of lactose. Strains with the ability to utilise citrate with production of diacetyl were classified as *S. diacetylactis* and subsequently as *Lc. lactis* subsp. *diacetylactis*. Because citrate utilisation is plasmid mediated it is an unstable characteristic of these bacteria, resulting in them being classified as a variety of *Lc. lactis* subsp. *lactis*. *Lc. lactis* subsp. *cremoris* are widely used in fermented dairy products. *Lc. lactis* subsp. *lactis* and subsp. *cremoris* are differentiated by the ability of subspecies *lactis* to grow at 40°C, in 4% NaCl, in 0.1% methylene blue milk and at pH 9.2 and to produce ammonia from arginine. Ribosomal RNA oligonucleotide probes have been developed to differentiate these subspecies (Klijn et al., 1991; Salama et al., 1991).

The use of lactococci is widespread and has the longest tradition in industrial starter culture technology. The principal concern of the dairy industry is the reliability and stability of these starter cultures. Many of the desirable traits of the lactococci are unstable because they are plasmid mediated. Extensive use of single strain cultures resulted in problems with bacteriophage. Genetic studies on lactococci have focused on the lactic fermentation, casein breakdown, diacetyl production from citrate and resistance to phage attack. Production of inhibitory substances (bacteriocins) by LAB is an area of increasing interest. Strains of *Lc. lactis* produce a range of bacteriocins the most important of which is the lantibiotic, nisin, which is a relatively broad spectrum bacteriocin that is active against gram-positive bacteria, including *Clostridium botulinum* and its spores. Nisin is licensed for use as a food preservative in over 45 countries (Delves-Broughton, 1990). Because a bacteriocin-producing strain could dominate the mixed cultures used for cheese making to the detriment of the fail-safe, multiple strain starter system, selection of starter strains has been against production of nisin or other antagonistic

substances. With the development of phage resistant starter strains, the use of single strain starter cultures is becoming a reality and bacteriocin production may well be viewed as an asset in dairy starter culture technology in the future.

## 4. The *Enterococcus* clade

### *4.1. The genus Enterococcus*

Bacteria of the genus *Enterococcus* have been recognised since Thiercelin (1899) described them as the 'entérocoque' to emphasise their intestinal origin and Andrewes and Horder (1906) used the name *S. faecalis* for the *Enterococcus*-type organism that was isolated from a patient with endocarditis. The taxonomy of this group of bacteria has been vague. There are no phenotypic characteristics that separate the genus from the other genera of gram-positive, catalase-negative cocci; in fact, phenotypic identification is generally by reverse identification (Devriese et al., 1993). The enterococci were described by Sherman (1937) as those organisms that grow at 10 and 45°C, in 6.5% NaCl and at pH 9.6, survive heating at 60°C for 30 min., and react with Lancefield group D antisera. Several species and strains of the genus *Enterococcus* do not meet all of these criteria (Devriese et al., 1993). Enterococci produce L( + )-lactic acid homofermentatively from glucose and also derive energy from degradation of amino acids. They have a PEP–PTS system for uptake of lactose and other carbohydrates, including gluconate (Bernsmann et al., 1982). There has been considerable disagreement about the generic relationships of the group D streptococci and the intra- and interspecific relationships of *S. faecalis*, *S. faecium* and *'S. durans'* (Farrow et al., 1983).

Sherman (1937) recognised that the *Enterococcus* division of his classification included the Lancefield group D streptococci. He suggested that they could be differentiated on the basis of haemolytic and proteolytic reactions; however, haemolysis is plasmid mediated (Ike et al., 1987) and therefore inappropriate as a characteristic for species differentiation. Application of genetic techniques to differentiate the enterococci has resolved many of the uncertainties about these bacteria. Kalina (1970) proposed the use of the generic name *Enterococcus* for *S. faecalis* and *S. faecium*. This was not accepted until the genus *Enterococcus* was revived by Schleifer and Kilpper-Bälz (1984) to include the Lancefield group D (faecal) streptococci, *S. faecalis* and *S. faecium*, as *E. faecalis* and *E. faecium*. This means that the group D antigen is not confined to the genus *Enterococcus* because *S. bovis* and *S. equinus* also produce the group D antigen. Since re-establishing the genus in 1984, a total of nine species has been transferred from the genus *Streptococcus* and ten new species have been added (Table 3).

Enterococci, usually *E. faecalis*, have been implicated as causal agents of human endocarditis, urinary tract and nosocomial infections (Kaye, 1982; Murray, 1990), whereas *E. faecium* is only implicated as the causative agent in 20% of enterococcal infections. They are frequently associated with polymicrobial infections of the abdomen and pelvis. Their aetiologic significance in these infections is not clear. Other species are seldom involved in human infections. The importance of the enterococci for food and public health microbiologists is related to their enteral habitat, their use as indicators for food safety and their possible involvement in foodborne illness (Stiles, 1989). The value of enterococci as indicators of faecal contamination of foods is limited by their ability to survive in the extraenteral environment, their relatively high heat resistance and the fact that they can dominate the microbial population of heat treated foods. Enterococci are also used as starter cultures in some foods and they are commercially available as probiotics for prevention and treatment of intestinal disorders of humans (Lewenstein et al., 1979) and animals (Ushe and Nagy, 1985). In particular, *E. faecium*, is associated with the fermentation of a number of southern European cheeses and is often applied in their processing.

In food microbiology it was considered important to identify *S. durans* to distinguish between group D streptococci of faecal and nonfaecal origin. *S. durans* has had a volatile taxonomic history ranging from species to varietal status within the group D streptococci. It was described

Table 3
Species included in the genus *Enterococcus*

| Species | Source | Principal habitats | Ref. |
|---|---|---|---|
| *E. faecalis* | | Human and other animal intestines | Schleifer and Kilpper-Bälz (1984) |
| *E. faecium* | | Human and other animal intestines, including poultry | Schleifer and Kilpper-Bälz (1984) |
| *E. avium* | Transfer | Poultry (rare) and mammalian intestines | Collins et al. (1984) |
| *E.casseliflavus*[a,b] | Transfer | Grass, silage, plants, soil | Collins et al. (1984) |
| *E. cecorum*[c] | Transfer | Clinical origin, animals | Williams et al. (1989) |
| *E. columbae* | New | Pigeon intestines | Devriese et al. (1990) |
| *E. dispar* | New | Human origin | Collins et al. (1991b) |
| *E. durans* | Transfer | Clinical isolates | Collins et al. (1984) |
| *E. fallox* | | New | |
| *E. flavescens*[a] | New | Clinical origin | Pompei et al. (1992) |
| *E. gallinarum*[b] | Transfer | Poultry intestines | Collins et al. (1984) |
| *E. hirae* | New | Animal intestines | Farrow and Collins (1985) |
| *E. mundtii*[a] | New | Grass, silage, plants, soil | Collins et al. (1986) |
| *E. malodoratus* | Transfer | Originally from Gouda cheese | Collins et al. (1984) |
| *E. pseudoavium* | New | Bovine mastitis | Collins et al. (1989c) |
| *E. raffinosus* | New | Clinical isolates, endocarditis | Collins et al. (1989c) |
| *E. saccharolyticus* | Transfer | Bedding and skin of cattle | Rodrigues and Collins (1990) |
| *E. seriolicida*[d] | New | | Kusuda et al. (1991) |
| *E. solitarius*[e] | New | | Collins et al. (1989c) |
| *E. sulfureus* | New | Plant material | Martinez-Murcia and Collins (1991b) |

New, new species; transfer, transfer from group D streptococci from Devriese and Pot (1995).
[a] Pigmented.
[b] Motile.
[c] Not group
[d] Syn. *Lact. garvieae* (?).
[d] Phylogenetically related to *Tetragenococcus*.

as a separate species by Sherman and Wing (1937), but it was included in *S. faecium* by Jones (1978). Based on DNA hybridisation studies it was shown that *E. faecium* consisted of two distinct entities (Farrow et al., 1983; Kilpper-Bälz et al., 1982), and the 'durans' group was proposed as *E. durans* (Collins et al., 1984). It is also listed as a pathogenic *Enterococcus*. *E. durans* has been isolated as an intestinal inhabitant of humans and poultry, discounting the previously held view that the durans variety represents a separate, nonfaecal population. It was also shown that *E. durans* consists of two distinct DNA homology groups. Homology group I has been retained as *E. durans*; homology group II is highly related to *E. hirae* that has been characterised among isolates from chicken and hog intestines (Farrow and Collins, 1985). *E. hirae* includes strains of human, bovine, avian and porcine origin (Knight and Shlaes, 1986) and it is primarily associated with chicken crops and hog intestines. It can be differentiated from other *Enterococcus* spp., including *E. durans*, by biochemical tests (Farrow and Collins, 1985). Some *Enterococcus* species, for example the pigmented species *E. mundtii* and *E. casseliflavus*, are primarily associated with plants. *E. faecalis* and *E. faecium* are widely distributed in the environment and they are also associated with plants (Martin and Mundt, 1972). Many of the newly described species of *Enterococcus* are of clinical origin, such as *E. durans* and *E. flavescens* (Pompei et al., 1992), whereas the pigmented species are primarily of plant origin. This means that public health and food microbiologists must still exercise caution when interpreting the meaning of enterococci in foods.

### 4.2. The genus Carnobacterium

This genus was proposed by Collins et al. (1987) and therefore it did not appear in 'Bergey's Manual of Systematic Bacteriology' published in 1986. Thornley (1957) reported the isolation of gram-positive, catalase-negative, non-sporeforming rods from poultry meat stored at low temperature. These bacteria resemble lactobacilli but they do not grow on acetate media. A similar group of bacteria isolated by Shaw and Harding (1984) from vacuum packaged, chill stored meats was referred to as the 'non-aciduric lactobacilli'. Two groups of non-aciduric lactobacilli had previously been described and proposed as the new species *Lb. divergens* (Holzapfel and Gerber, 1983) and *Lb. carnis* (Shaw and Harding, 1985). Originally thought to be heterofermentative, these organisms were shown to follow the glycolytic pathway of glucose fermentation, with small amounts of $CO_2$ being produced by some form of endogeneous metabolism (De Bruyn et al., 1988). Because these two species were not included in earlier comparative studies, Collins et al. (1987) studied them in association with *Lb. piscicola*, the pathogen of salmonid fish (Hiu et al., 1984), which shares many properties with the nonaciduric (atypical) lactobacilli from meat. The majority of the poultry strains were assigned to *Brochothrix thermosphacta*, *Lb. divergens* and *Lb. piscicola* (Collins et al., 1987). Based on their phenotypic characteristics, the new genus *Carnobacterium* was proposed (Table 4). *C. gallinarum* and *C. mobile* incorporate the poultry strains described by Thornley (1957).

Comparative 16S rRNA sequence analysis studies of the genus *Carnobacterium* (Wallbanks et al., 1990) confirmed the similarity of these organisms and their distinction from all other LAB. Although the carnobacteria were originally isolated with the lactobacilli, phylogenetically the genus is more closely related to the genera *Enterococcus* and *Vagococcus*. *C. piscicola* (*Lb. piscicola*, Hiu et al., 1984) shows 100% rRNA sequence homology with *Lb. maltaromicus* (Miller et al., 1974), hence Collins et al. (1991a) proposed that the correct name for this organism should be *C. maltaromicus*. Two additional species, proposed by Franzmann et al. (1991), *C. funditum* and *C. alterfunditum*, were isolated from lake water in Antarctica. They are motile bacteria with a subpolar flagellum and they are most closely related to *C. mobile*. The food-associated carnobacteria have a number of physiological features in common with the enterococci (and not with the lactobacilli), notably their ability to grow at pH 9.5, resistance to thallous acetate, antibiotic resistance and vitamin requirements. Only sparse information is available on the association of carnobacteria with foods other than meat, poultry and fish. *C. piscicola* and *C. divergens* have been reported to dominate the microflora of mould-ripened soft white cheese (Milliére et al., 1994). The 'original' habitat of these species is still unknown.

### 4.3. The genus Tetragenococcus

*P. halophilus* is important in the fermentation of soy moromi to produce soy sauce. It requires NaCl for growth and it grows in 18% NaCl. In phylogenetic studies of the genus *Pediococcus* using 16S rRNA sequence analysis (Collins et al., 1990) it was shown that *P. halophilus* is clearly separated from the other pediococci, and a new genus *Tetragenococcus* was proposed for this organism. It is more closely related to *Enterococcus* and *Carnobacterium* than to *Lactobacillus*.

Table 4
*Carnobacterium* species, their relationship to previously described bacteria and their habitat (from Collins et al., 1987, 1991a)

| Current nomenclature | Previous nomenclature | Habitat |
|---|---|---|
| *C. divergens* | *Lb. divergens* | Meat, poultry, surface ripened mould cheeses |
| *C. gallinarum* | | Poultry |
| *C. mobile* | | Poultry |
| *C. piscicola*[a] | *Lb. piscicola* | Meat, poultry, salmonid fish |
| | *Lb. carnis* | |
| | *Lb. maltaromicus* | |
| *C. funditum* | | Antarctic lake |
| *C. alterfunditum* | | Antarctic lake |

[a] Proposed as *C. maltaromicus* (Collins et al., 1991a).

### 4.4. The genus *Vagococcus*

Studies using 16S rRNA sequences established that the motile group N streptococci are more closely related to the genus *Enterococcus*, the carnobacteria and *Listeria* than to the genera *Streptococcus* and *Lactococcus* (Collins et al., 1989a, 1993a; Devriese et al., 1993). The motile group N streptococci isolated from chicken faeces and river water are now designated as *Vagococcus fluvialis* (Collins et al., 1989a). A new species *V. salmoninarum* was isolated from diseased salmonid fish (Wallbanks et al., 1990).

## 5. The genera *Lactobacillus*, *Leuconostoc* and *Pediococcus*

In the past these genera were considered distinct with little possibility of overlap or interrelationship. Chemotaxonomic and phylogenetic studies have shown that this is not the case and that there is indeed significant overlap between all three of these genera that do not follow either morphological or previous physiological lines of division. The principal groupings based on 16S rRNA studies are summarised as follows (Collins et al., 1991a): (1) *Lb. delbrueckii* group including primarily, but not exclusively, the homofermentative lactobacilli; (2) the *Lb. casei–Pediococcus* group, comprised of obligate homofermenters as well as facultative and obligate heterofermenters; (3) the *Leuconostoc* group that includes some obligately heterofermentative lactobacilli and has subsequently been subdivided into three genera: *Leuconostoc*, *Oenococcus* and *Weissella*. This phylogenetic subdivision is shown diagrammatically in Fig. 2.

### 5.1. The genus *Lactobacillus*

The classical division of the lactobacilli was based on their fermentative characteristics: (1) obligately homofermentative; (2) facultatively heterofermentative; and (3) obligately heterofermentative. This division suited the interests of food microbiologists. Several lactobacilli of groups 1 and 2 and some of the heterofermentative group 3 lactobacilli are either used in fermented foods, but group 3 are also commonly associated with food spoilage. In Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986) the genus *Lactobacillus* was described with a heterogeneous group of 'regular non-sporing gram-positive rods.' Four of the seven genera are facultative anaerobes, of which *Lactobacillus* and *Erysipelothrix* are catalase negative and *Brochothrix* and *Listeria* catalase positive. The four genera are important in foods, but they represent unrelated groups of bacteria, including those associated with: (1) food fermentation, *Lactobacillus*; (2) food spoilage, *Brochothrix* and *Lactobacillus*; and (3) potential or established foodborne infections, *Erysipelothrix* and *Listeria*. The genus *Lactobacillus* is heterogeneous with 33–55 mol% G + C content in the DNA (Collins et al., 1991a; Hammes and Vogel, 1995). Generally, it is suggested that not more than 10% range in G + C content should exist in a well defined genus (Vandamme et al., 1996).

With the development of phylogenetic analysis in the 1980s there have been many changes to the genus *Lactobacillus*. *Lb. catenaformis*, *Lb. rogosae* and *Lb. vitulinus* (Stackebrandt and Teuber, 1988) and several other former species are no longer members of the genus. *Lb. minutus*, *Lb. uli* and *Lb. rimae* are related to each other and to *S. parvulus*, justifying their classification in a new genus *Atopobium* that is more closely related to the bifidobacteria than the lactic acid bacteria (Collins and Wallbanks, 1992). *Lb. maltaromicus* is now *C. piscicola* and currently proposed as *C. maltaromicus* (Collins et al., 1991a) and *Lb. carnis* and *Lb. divergens* were transferred to the new genus *Carnobacterium* (Collins et al., 1987). Pot et al. (1994) ably documented the LAB and their valid descriptions, including the names and synonyms for the lactobacilli. Homologies within the genus have resulted in the following synonyms being recognized and incorporated into the nomenclature of the lactobacilli: *Lb. murinus* as a synonym for *Lb. animalis* (Dent and Williams, 1982), *Lb. sake* for *Lb. bavaricus* (Kagermeier-Callaway and Lauer, 1995), *Lb. helveticus* for *Lb. jugurti* (Dellaglio et al., 1973), *Lb. mali* for *Lb. yamanashiensis* (Carr and Davies, 1970), *Lb. fermentum* for *Lb. cellobiosus* (Vescovo et al., 1979)

Table 5
Habitats of the genus *Lactobacillus*

| |
|---|
| Humans |
| Oral cavity |
| Intestinal tract |
| Vagina |
| Other habitats |
| Plants and plant materials |
| Soil, water, sewage and manure |
| Food fermentations (milk, meat and vegetable) |
| Cereal products |
| Silage |
| Food spoilage |
| Beer |
| Fruit and grain mashes |
| Marinated fish |
| Sugar processing |
| Milk |
| Meat and meat products |
| Fermented beverages |

and *Lb. fructivorans* for *Lb. trichodes* (Weiss et al., 1983a). New species (*Lb. kefirgranum* and *Lb. parakefir*) from kefir grains (Takizawa et al., 1994) and sourdough bread (*Lb. pontis* and *Lb. panis*) (Vogel et al., 1994; Wiese et al., 1996) have been described since the listing by Pot et al. (1994). The present number of *Lactobacillus* spp. remains greater than 50, despite the transfer of five heterofermentative species of *Lactobacillus* to the new genus *Weissella* by Collins et al. (1993b).

The lactobacilli are strictly fermentative and have complex nutritional requirements. They grow in and are associated with many different habitats (Table 5). They are aciduric or acidophilic, producing pH 4.0 in foods containing a fermentable carbohydrate. As a result, they often suppress growth or kill other bacteria. It is generally accepted that lactobacilli grow up to a maximum pH of 7.2, although exceptions with respect to substrate and strain exist. Lactobacilli are used as starter cultures for several varieties of cheese, fermented plant foods, fermented meats, in wine and beer production, sourdough bread and silage. Unlike the coccus-shaped lactics, the lactobacilli were not separated into different genera based on their homo- or heterofermentation of hexoses. The current taxonomic status of the lactobacilli based on the classical phenotypic subdivision shown in Table 6 was derived from the reviews by Hammes et al. (1991), Pot et al. (1994) and Vandamme et al. (1996).

Group 1 includes the obligately homofermentative lactobacilli that ferment glucose exclusively to lactic acid and do not ferment pentoses or gluconate. It represents Orla-Jensen's thermobacteria and the important food associated species: *Lb.*

Table 6
Major divisions within the genus *Lactobacillus* based on phenotypic characteristics

| Group 1 | Group 2 | Group 3 |
|---|---|---|
| Obligate homofermenters | Facultative heterofermenters | Obligate heterofermenters |
| ***Lb. acidophilus*** | ***Lb. acetotolerans*** | ***Lb. brevis*** |
| *Lb. amylophilus* | *Lb. agilis* | ***Lb. buchneri*** |
| *Lb. amylovorus* | ***Lb. alimentarius*** | ***Lb. collinoides*** |
| *Lb. aviarius* | ***Lb. bifermentans*** | ***Lb. fermentum*** |
| subsp. *araffinosus* | ***Lb. casei*** | ***Lb. fructivorans*** |
| subsp. *aviarius* | *Lb. coryniformis* | *Lb. fructosus*[b] |
| *Lb. crispatus* | subsp. *coryniformis* | ***Lb. hilgardii*** |
| ***Lb. delbrueckii*** | subsp. *torquens* | ***Lb. kefir*** |
| subsp. ***bulgaricus*** | ***Lb. curvatus*** | ***Lb. malefermentans*** |
| subsp. ***delbrueckii*** | *Lb. graminis* | *Lb. oris* |
| subsp. ***lactis*** | *Lb. hamsteri* | ***Lb. panis***[a] |
| ***Lb. farciminis*** | ***Lb. homohiochii*** | ***Lb. parabuchneri*** |
| *Lb. gallinarum* | *Lb. intestinalis* | ***Lb. parakefir***[a] |
| ***Lb. gasseri*** | *Lb. murinus* | ***Lb. pontis***[a] |
| ***Lb. helveticus*** | ***Lb. paracasei*** | ***Lb. reuteri*** |
| *Lb. jensenii* | subsp. ***paracasei*** | ***Lb. sanfrancisco*** |
| ***Lb. johnsonii*** | subsp. ***tolerans*** | ***Lb. suebicus*** |
| ***Lb. kefiranofaciens*** | ***Lb. paraplantarum***[a] | ***Lb. vaccinostercus*** |
| ***Lb. kefirgranum***[a] | ***Lb. pentosus*** | ***Lb. vaginalis*** |
| ***Lb. mali*** | ***Lb. plantarum*** | |
| *Lb. ruminis* | ***Lb. rhamnosus*** | |
| *Lb. salivarius* | ***Lb. sake*** | |
| subsp. *salicinus* | | |
| subsp. *salivarius* | | |
| *Lb. sharpeae* | | |

Data collected by Hammes and Vogel (1995), Pot et al. (1994) and Vandamme et al. (1996). Bold face, lactobacilli of importance in foods and as probiotics.
[a] Species added since the review by Pot et al. (1994).
[b] *Lb. fructosus* classified with the *Leuconostoc* group of lactic acid bacteria.

Table 7
Phenotypic features of the species within the *Lactobacillus acidophilus* 'group' (modified after Mitsuoka, 1992)

| Species | Homology group | Occurrence | mol% G+C in the DNA | Fermentation of | | | Growth in NaCl (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Melibiose | Raffinose | Trehalose | 3.5 | 4.5 | 5 |
| *Lb. acidophilus* | A-1[a]/Ia[b] | ? | 32–37 | – | V | + | – | – | – |
| *Lb. amylovorus* | A-3[a]/Ib[b] | S/C | 40 | V | + | + | – | – | – |
| *Lb. crispatus* | A-2[a]/Ic[b] | M/P | 35–38 | + | + | – | + | – | – |
| *L. gallinarum* | A-4[a]/Id[b] | P | 33–36 | + | + | – | + | + | – |
| *Lb. gasseri* | B-1[a]/IIa[b] | M/C | 33–35 | – | – | + | + | + | V |
| *Lb. johnsonii* | B-2[a]/IIb[b] | M/S/P | 32–38 | V | V | + | + | + | + |

M, man; S, swine; C, cattle; P, poultry.
[a] Source: Johnson et al. (1980).
[b] Source: Lauer et al. (1980).

*acidophilus*, *Lb. delbrueckii* and *Lb. helveticus*, as well as *Lb. farciminis* and *Lb. kefiranofaciens*.

*Lb. delbrueckii* forms an important complex of bacteria that previously had species status as *Lb. delbrueckii*, *Lb. bulgaricus*, *Lb. lactis* and *Lb. leichmanii*. They have 80% DNA homology and have therefore been reclassified as *Lb. delbrueckii* subspp. *delbrueckii*, *bulgaricus* and *lactis* (Weiss et al., 1983b). *Lb. leichmanii* has been incorporated with *Lb. delbrueckii* subsp. *lactis*. All of these organisms are associated with plant and dairy foods that are fermented at high temperatures of 45–50°C. *Lb. delbrueckii* subsp. *bulgaricus* is a starter strain for yoghurt manufacture (see *S. thermophilus* earlier). *Lb. delbrueckii* subsp. *bulgaricus* is also used in association with *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus* and *S. thermophilus* as starter cultures for Swiss-type Emmental and Gruyére cheeses and Italian-type Gorgonzola, Mozzarella, Provolone and Caciocavallo cheeses (Hammes et al., 1991). *Lb. delbrueckii* subsp. *delbrueckii* may be associated with the fermentation of sourdough bread.

The original isolation of *Lb. acidophilus* in 1900 was from infant faeces but strains reported as *Lb. acidophilus* based on biochemical characteristics were shown to be heterogeneous on the basis of DNA–DNA homology (Lauer et al., 1980). They were divided into two main genotypic groups designated A and B (Johnson et al., 1980), or 1 and 2 (Lauer et al., 1980). Altogether six homology groups within the *Lb. acidophilus*-group have been proposed as valid species (Table 7). These species cannot be differentiated by simple phenotypic tests, although a number of growth features may be useful for their characterisation (Mitsuoka, 1992). *Lb. acidophilus* and *Lb. gasseri* can be differentiated by 23S rRNA oligonucleotide probes (Pot et al., 1993). *Lb. acidophilus* is used in the production of acidophilus milk. It is considered as important representative of probiotic bacteria, although these are not restricted to *Lb. acidophilus sensu stricto* (Gasser and Mandel, 1968) or other species of the *Lb. acidophilus* homology group (Johnson et al., 1980; Lauer et al., 1980; Cato et al., 1983). *Lb. acidophilus* BG2 FO4 that was studied by Kleeman and Klaenhammer (1982) has been identified as *Lb. johnsonii* (Fujisawa et al., 1992). This strain adheres to human foetal intestinal cells *in vitro*, indicating its ability of adherence to epithelial cells in vivo. Especially *Lb. johnsonii* appears to be typically used in novel type yoghurts with claims on their healthfulness (Holzapfel et al., 1996). This species and other of the *Lb. acidophilus*-group are currently being studied for their typical 'probiotic' properties. To avoid the probiotic potential of these bacteria falling once again into disrepute, reliable basic information on microbial antagonism, survival of the stomach passage, growth and metabolic behaviour in the small and large intestines and attachment to the epithelial cells of the gut is required.

*Lb. helveticus* is considered closely related to *Lb. acidophilus* (Yang and Woese, 1989; Collins et al., 1991a) and was established as synonymous with *Lb. jugurti* (Pot et al., 1994). *Lb. helveticus* is distinct from *Lb. delbrueckii* and is an important organism in Swiss- and Italian-type cheeses. *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. acidophilus* and *Lb. farciminis* are obligately homofermentative lactobacilli associated with the fermentation of sourdough bread. *Lb. kefiranofaciens* is an important organism associated with kefir grains. It produces the kefiran polymer that forms the matrix of the kefir grains. Other bacteria associated with kefir are *Lb. acidophilus*, the obligately heterofermentative *Lb. kefir* and the yeast *Candida kefir* (Toba et al., 1986). A study of *Lactobacillus* isolates from kefir grains (Takizawa et al., 1994) resulted in the description of a new homofermentative species *Lb. kefirgranum* and a new heterofermentative species *Lb. parakefir*.

Group 2 includes the facultatively heterofermentative lactobacilli that ferment hexoses to lactic acid and may produce gas from gluconate but not from glucose. They also ferment pentoses by an inducible phosphoketolase to produce lactic and acetic acids. Important food-associated species in this group include *Lb. casei* and *Lb. plantarum*.

*Lb. casei* is associated with many habitats, including dairy products, silage, the human mouth and intestine and sewage. It is specifically associated with sourdough bread and some brined cheese fermentations and it can cause spoilage of cheeses by fermentation of citrate to release carbon dioxide (Hammes et al., 1991). The species was poorly defined and contained five subspecies based on phenotypic characteristics. Studies of DNA homology by Collins et al. (1989b) indicated that the majority of organisms designated *Lb. casei* subsp. *casei*, together with *Lb. casei* subsp. *alactosus*, *pseudoplantarum* and *tolerans*, have high levels of DNA relatedness but they are reported to be distinct from the type strain of *Lb. casei* subsp. *casei*. Collins et al. (1989b) proposed that this homology group be given species status as *Lb. paracasei* with subspecies *paracasei* to contain all of the subspecies, except subspecies *tolerans* which was proposed as *Lb. paracasei* subsp. *tolerans*. Furthermore, it was proposed that *Lb. casei* subsp. *rhamnosus* should be elevated to species rank as *Lb. rhamnosus*. The close relationship between these strains was confirmed by 16S RNA sequence homology (Collins et al., 1991a); however, the species name *Lb. paracasei* was questioned by Dellaglio et al. (1991), since the type strain (ATCC 393) of *Lb. casei* ssp. *casei* shows only low levels of DNA homology with other strains of *Lb. casei* ssp. *casei* and of *Lb. paracasei* ssp. *paracasei*. On the other hand, strain ATCC 393 exhibits high DNA homology with *Lb. rhamnosus* ATCC 15820, which was the original 'type strain' of *Lb. zeae* Kuznetsov, 1959, isolated from corn steep liquor. After initial rejection of their request, Dicks et al. (1996) now finally recommended the reclassification of *Lb. casei* ssp. *casei* ATCC 393 and *Lb. rhamnosus ATCC 15820* as *Lb. zeae* and strain ATCC 334 as the neotype strain of *Lb. casei* ssp. *casei*. They also recommended the rejection of the species name *Lb. paracasei*.

*Lb. plantarum* is used as a starter organism in some fermented sausages and cereal products; it is a part of the adventitious LAB growing in fermented vegetable and meat products and it is a spoilage organism in citrus juice, wine and some cheeses (Hammes et al., 1991). It was considered an important organism in the natural fermentation of meats, although this assumption may be based on the false classification of atypical streptobacteria. In North America its use as a meat starter culture has been superseded by *P. acidilactici* and in Europe mainly by *Lb. curvatus* and *Lb. sake*. *Lb. plantarum* was classified with Orla-Jensen's streptobacteria because the strains studied were not able to grow at 45°C. Although some strains have been reported to grow at 45°C, the general ability to grow at 15°C serves as confirmation for the allocation of *Lb. plantarum* to the streptobacteria. It has been a 'catch-all' species for atypical lactobacilli. The strains classified as *Lb. plantarum* include two DNA homology groups. *Lb. plantarum* and *Lb. pentosus* have long been considered synonymous, but Zanoni et al. (1987) revived the presently valid species name *Lb. pentosus*. Collins et al. (1991a) reported high 16S rRNA sequence similarity between these spe-

cies, confirming that they are closely related. Facultatively heterofermentative lactobacilli with similar physiological characteristics to *Lb. plantarum* isolated as beer contaminants and from faeces were tested for their relatedness to *Lb. plantarum* and *Lb. pentosus*. Based on the lack of DNA relatedness, a new species *Lb. paraplantarum* has been proposed (Curk et al., 1996).

A third subgroup of the group 2 lactobacilli includes *Lb. curvatus* and *Lb. sake* which have important associations with foods. Most strains of *Lb. bavaricus* that produce L(+)-lactate show high DNA homology with *Lb. sake* and some with *Lb. curvatus*, and they are considered racemase-defective variants of these bacteria. Because of heterogeneity within these two species, two subgroups for each have been suggested (Klein et al., 1996). These bacteria are an important part of the adventitious microflora of modified atmosphere and vacuum packaged meats and meat products that are stored at refrigeration temperatures ($< 5°C$) and they are beneficial in sauerkraut, brined fruit and vegetable fermentations. *Lb. curvatus* and *Lb. sake* are important starter cultures in fermented meat products (Hammes et al., 1991; Vogel et al., 1993) and typically dominate the microbial population of these products. Sulphide-producing strains of *Lb. sake* have been implicated in spoilage of chill stored, vacuum packaged meat (Egan et al., 1989). Co-culture of bacteriocinogenic *Leuc. gelidum* and sulphide-producing *Lb. sake* on chill stored, vacuum packaged beef resulted in inhibition of sulphide spoilage (Leisner et al., 1996). *Lb. sake* (comprising ca. 60%) and *Lb. curvatus* (with ca. 20%) were found to dominate the spoilage association of vacuum packaged Frankfurter, Vienna and related sausage types (German, 'Brühwurst'), causing sliminess, exudation, gas-production and off-flavours (Holzapfel and Gerber, 1986). They were also shown to be the major cause of ropiness in Finnish ring sausages (Korkeala et al., 1988; Mäkelä et al., 1992). The type strain of *Lb. homohiochii* that is part of the LAB microflora of sourdough bread is closely related to *Lb. sake* (Dellaglio et al., 1975). DNA:DNA hybridisation studies of the species in this subgroup have shown that they are closely related, but their species status has been maintained. Particular atypical streptobacteria, designated non-aciduric lactobacilli, were described by Shaw and Harding (1984) as an important group of bacteria growing in vacuum packaged meats (see genus *Carnobacterium*).

Group 3 includes the obligately heterofermentative lactobacilli that ferment hexoses to lactic acid, acetic acid and/or ethanol and carbon dioxide. The production of gas from glucose is a characteristic feature of these bacteria. The most important obligate heterofermentative *Lactobacillus* associated with food fermentations is *Lb. sanfrancisco* which converts maltose to lactic and acetic acids and various flavour compounds in sourdough bread. New species of lactobacilli associated with the rye sourdough fermentation have been described as *Lb. pontis* (Vogel et al., 1994) and *Lb. panis* (Wiese et al., 1996). *Lb. brevis* and *Lb. fermentum* are also associated with lactic fermentations of sour dough. *Lb. kefir* is one of the bacteria involved with kefir grains (Hammes et al., 1991). Among the heterofermentative lactobacilli, *Lb. reuteri* has attracted considerable interest because of its antimicrobial metabolite 'reuterin' (3-hydroxypropionaldehyde) that is active against a broad spectrum of microorganisms. A commercial milk product based on *L. reuteri* (BRA-milk) was introduced in Sweden in 1991 (Ståhl and Molin, 1994). *Lb. reuteri* and *Lb. fermentum* are difficult to distinguish by conventional physiological tests, but genetically they are not closely related, as indicated by the difference in mol% G+C of the DNA (40–42 and 52–54, respectively), and ornithine in the peptidoglycan of *Lb. fermentum* (Kandler and Weiss, 1986). Some lactobacilli, especially among the group III, cause spoilage of foods, such as *Lb. bifermentans* that causes cracking defects in Gouda and Edam cheeses due to gas production, *Lb. brevis* that causes spoilage of citrus fruit, wines and beer, *Lb. fructivorans*, *Lb. brevis* and *Lb. buchneri*, causing spoilage of mayonnaise dressings and acetic acid (vegetable) preserves (Baumgart, 1965; Dakin and Radwell, 1971) and *Lb. viridescens* (now *Weissella viridescens*) that causes greening of cured meat products (Hammes et al., 1991). The heterofermentative lactobacilli that lack aspartic acid or

Table 8
*Leuconostoc* branch of the lactobacilli showing the subgroupings reported by Collins et al. (1991) and *Oenococcus* (Dicks et al., 1995)

| Listed in Bergey's Manual (1986) | New species established since 1986 | Ref. |
|---|---|---|
| ***Leuc. mesenteroides*** | *Leuc. carnosum*[a] | Shaw and Harding, 1989 |
| **subsp. *mesenteroides*** | *Leuc. gelidum* | Shaw and Harding, 1989 |
| **subsp. *cremoris*** | *Leuc. citreum* | Farrow et al., 1989 |
| **subsp. *dextranicum*** | *Leuc. pseudomesenteroides* | Farrow et al., 1989 |
| ***Leuc. lactis*** | (*Leuc. amelibiosum*)[b] | Schillinger et al., 1989 |
| | *Leuc. argentinum* | Dicks et al., 1993 |
| | *Leuc. fallax* | Martinez-Murcia and Collins, 1991a |
| ***Lactobacillus fructosus*** | | |
| ***Leuc. oenos*** reclassified as | *Oenococcus oeni* | Dicks et al., 1995 |

Organisms listed in bold type are those listed in Bergey's Manual (Sneath et al., 1986).
[a] Newly described species.
[b] 100% homology with *Leuc. citreum* (Takahashi et al., 1992).

diaminopimelic acid in their peptidoglycan appear phylogenetically different from other lactobacilli and were grouped with *Leuc. paramesenteroides* into a new genus *Weissella* (Collins et al., 1993b).

### 5.2. The genus *Leuconostoc*

The original classification of bacteria was largely based on morphology. This placed the genus *Leuconostoc* close to the streptococci, albeit in a separate genus as the 'heterofermentative cocci' formerly called the betacocci by Orla-Jensen. They produce D(−) lactate from glucose as opposed to L(+)-lactate that is produced by the lactococci and DL-lactate by the heterofermentative lactobacilli with whom, they share many characteristics. *Leuconostoc* is the predominant genus among the LAB on plants, with *Leuc. mesenteroides* subsp. *mesenteroides* as the principal isolate (Mundt, 1970). On living, undamaged plant tissue they occur in relatively low numbers, but as the plant matures and during harvesting and ensiling their numbers increase (Daeschel et al., 1987). In fermented foods of plant origin, *Leuc. mesenteroides* is generally the first organism to grow and it is succeeded by the more acid-tolerant lactobacilli (Stamer, 1975). The *Leuconostoc* group has considerable species-specific commercial importance (Garvie, 1984), including spoilage in sugar processing by production of dextrans, the malolactic fermentation (by *Oenococcus*) in wine making, production of flavour components from citrate in dairy fermentations and production of dextrans that have wide applications in research, industry and medicine (Holzapfel and Schillinger, 1991).

In Bergey's Manual of Systematic Bacteriology the number of species in the genus *Leuconostoc* was reduced from six to four. The previous species *Leuc. mesenteroides*, *dextranicum* and *cremoris* were all included as subspecies of *Leuc. mesenteroides* (Garvie, 1986a). *Lb. fructosus*, *Lb. viridescens* and *Lb. minor* share key characteristics with the leuconostocs, including absence of arginine dehydrolase and similar peptidoglycan composition (Garvie, 1986a). Yang and Woese (1989) reported a phylogenetic group comprised of the leuconostocs and several lactobacilli. They described this as the leuconostoc branch of the lactobacilli. Another species *Leuc. amelibiosum* was isolated from sugar solutions in refineries (Schillinger et al., 1989) and was originally described as *Leuc. mesenteroides* subsp. *amelibiosum* (Holzapfel, 1969). This species does not grow at 5°C, it produces dextran from sucrose and causes spoilage in sugar refining. Two other *Leuconostoc* species, *Leuc. citreum* and *Leuc. pseudomesenteroides*, were identified from clinical isolates (Farrow et al., 1989). Changes to the genus are shown in Tables 8 and 9. In recent years the LAB (including leuconostocs) that grow in chill stored meats have attracted considerable attention because of their prevalence in raw and processed meats packaged under vacuum or in modified

atmosphere with increased carbon dioxide (Cavett, 1963; Savell et al., 1981; Shaw and Harding, 1984). From a numerical taxonomic study of 52 strains of *Leuconostoc* spp. isolated from chill stored meats, Shaw and Harding (1989) identified three clusters. One cluster was identified as *Leuc. mesenteroides* subsp. *mesenteroides;* the other two clusters were shown to represent two new species for which the names *Leuc. gelidum* and *Leuc. carnosum* were proposed (Table 8). *Leuc. gelidum* has the potential for a biopreservative role in fresh meats (Leisner et al., 1996).

*Leuconostoc* comprises three distinct evolutionary lines or clusters (Yang and Woese, 1989; Martinez-Murcia and Collins, 1990). The main cluster has been designated *Leuc. sensu stricto* and it is based on 97–99% rRNA sequence homology. It has been differentiated into three subgroups (Martinez-Murcia and Collins, 1990): (i) *Leuc. mesenteroides* and *Leuc. pseudomesenteroides*; (ii) *Leuc. lactis* and *Leuc. citreum*; and (iii) the meat isolates *Leuc. carnosum* and *Leuc. gelidum*. *Leuc. amelibiosum* (Schillinger et al., 1989) has been assigned to *Leuc. citreum* based on phenotype and DNA relatedness studies (Takahashi et al., 1992). *Lb. fructosus* has also been assigned to *Leuc. sensu stricto* (Collins et al., 1991a). The atypical leuconostoc-like organism isolated from sauerkraut by Schillinger et al. (1989) has 94–96% homology with *Leuc. sensu stricto* and represents a new species designated *Leuc. fallax* (Martinez-Murcia and Collins, 1991a). In a study of *Leuconostoc* spp. isolated from raw milk in Argentina, 7–10% of the isolates had low levels of DNA homology with other leuconostocs and they were proposed as a new species *Leuc. argentinum* (Dicks et al., 1993).

Table 9
Assignment of *Lactobacillus* and *Leuconostoc* species to the new genus *Weissella* (Collins et al., 1993a)

| Paramesenteroides group | New designation as genus *Weissella* (Collins et al., 1993a) |
|---|---|
| ***Leuc. paramesenteroides*** | *W. paramesenteroides* |
| ***Lb. confusus*** | *W. confusa* |
| ***Lb. halotolerans*** | *W. halotolerans* |
| ***Lb. kandleri*** | *W. kandleri* |
| ***Lb. minor*** | *W. minor* |
| ***Lb. viridescens*** | *W. viridescens* |
| | *W. hellenica* sp. nov.[a] |

Organisms listed in bold type are those listed in Bergey's Manual (Sneath et al., 1986).
[a] Newly described species by Collins et al. (1993a).

### 5.3. The genus Pediococcus

As a result of their association with beer spoilage, the pediococci were among the first bacteria to be studied by Louis Pasteur. Tetrad formation and spherical shape served as key characteristics for their early recognition. They were the only LAB that divide in two planes to produce tetrads or pairs, but taxonomic changes have increased the number of tetrad forming genera to three. Pediococci are most likely to be confused with micrococci because of morphological similarities, pseudocatalase production and salt tolerance (Garvie, 1986b) and also with the aerococci. The pediococci are homofermentative and, with the exception of *L. dextrinicus* which produces L(+)-lactic acid, all species produce DL – lactate from glucose. The pediococci of beer and plant origin were included in one species as *P. cerevisiae*, but studies on isolates from these two sources were shown to be different and they were assigned to *P. damnosus* and *P. pentosaceus*, respectively (Raccach, 1987). Most of the strains designated *P. cerevisiae* that were used as meat starters have been reclassified as *P. acidilactici*. In Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986) eight species are recognised (Table 10). The genus *Pediococcus* was shown to be phylogenetically heterogeneous (Collins et al., 1990). The type strain *Pediococcus damnosus* forms a closely related group with *P. acidilactici*, *P. parvulus* and *P. pentosaceus*. Despite their morphological distinctiveness, Collins et al. (1991a) clearly demonstrated a relationship between the pediococci and lactobacilli of the *Lb. casei* group. *P. acidilactici*, *P. damnosus*, *P. parvulus* and *P. pentosaceus* form an evolutionary grouping with *Lb. pentosus*, *Lb. brevis* and the *Lb. buchneri* complex. *P. dextrinicus* is related but peripheral to other members of the genus *Pediococcus* and

Table 10
Species of *Pediococcus* and related tetrad-forming bacteria and their common habitats

| Bacterial name | Habitat |
|---|---|
| *P. damnosus* | Breweries (beer cloudiness); wine and cider |
| *P. dextrinicus* | Beer, silage |
| *P. parvulus* | Sauerkraut, silage |
| *P. inopinatus* | Sauerkraut, beer |
| *P. pentosaceus* | Vegetable material, fermented sausages, milk and dairy products |
| *P. acidilactici* | Vegetable material, milk and dairy products |
| *Aerococcus urinae-equi*[a] | Not associated with foods |
| *Tetragenococcus halophilus*[b] | Soy sauce, pickling brines (requires salt for growth) |

[a] Previously *P. urinae-equi.*
[b] Previously *P. halophilus.*

shares phylogenetic relatedness to *Lb. coryniformis* and *Lb. bifermentans* (Collins et al., 1990, 1991a).

Pediococci are most often found in low numbers, together with leuconostocs and lactobacilli, on plant materials, in various foods and as spoilage agents in alcoholic beverages such as beer. The pediococci are important starter bacteria in fermented sausages of some regions. Some of the widely used starter strains such as *P. acidilactici* and *P. pentosaceus* produce bacteriocins. Although they are of economic importance as starter cultures, not all of the fermentation pathways utilised by pediococci are clear. They require a fermentable carbohydrate for growth and grow poorly in milk because lactose is not readily utilised. Fermentation of glucose follows the Embden–Meyerhof pathway with DL- or L-(+)-lactate as the major end product under optimal conditions. Pyruvate can be diverted to other end products and diacetyl/acetoin is often produced by *P. damnosus*, while *P. pentosaceus* produces equimolar amounts of lactate and acetate from pentoses (Fukui et al., 1957).

Some species exhibit extreme tolerances to temperature, pH and NaCl. For example, *P. acidilactici* grows at 50°C and is heat tolerant; *P. damnosus* and *P. parvulus* are acid and hop tolerant and grow at low temperatures but generally require more anaerobic growth conditions than other strains. Production of diacetyl is diagnostic for *P. damnosus*, but under growth limiting conditions acetoin and/or diacetyl can be produced. *P. pentosaceus* and *P. acidilactici* are closely related species that may not be clearly differentiated by phenotypic characteristics, but they are differentiated by DNA–DNA homology (Garvie, 1986b). *P. halophilus* grows in 18% NaCl, but it has been transferred to a new genus *Tetragenococcus* that is more closely related to the enterococci and carnobacteria than to the pediococci and lactobacilli (*vide supra*). Strains belonging to *P. urinae-equi* have been transferred to *Aerococcus.*

### *5.4. Phylogenetic associations within the genera Lactobacillus, Leuconostoc and Pediococcus*

Hybridisation studies of Schillinger et al. (1989) on *Leuconostoc* and heterofermentative lactobacilli indicated that *Leuc. paramesenteroides* is more closely related to *Lb. confusus*, *Lb. halotolerans*, *Lb. kandleri*, *Lb. minor* and *Lb. viridescens* than to *Leuc. sensu stricto.* In a study of the LAB isolated from fermented Greek sausage, taxonomic studies on *Leuconostoc*-like organisms led Collins et al. (1993a) to propose that *Leuc. paramesenteroides* and related species should be reclassified in a new genus *Weissella.* They also described a new species, *Weissella hellenica*, for the isolates obtained from fermented sausage.

Strains of *Leuconostoc* from wine that grew on acidic media were classified as *Leuc. oenos* (Garvie, 1967). It is represented by strains that are typically acidophilic, grow in grape must and wine at pH 3.5 and are not inhibited by 10% ethanol. They are responsible for the malolactic fermentation of wines and cider, in which L-malate is converted to L(+)-lactate and $CO_2$, otherwise D(−)-lactate is produced from glucose by all *Leuconostoc* spp. The malolactic fermentation produces desired flavour attributes or it can result in defects of haziness, gassiness and sedimentation in wines. Commercial strains are available for use in wine production. Physiological differences of *Leuc. oenos* from other leuconostocs, including growth at initial pH 4.8 and in media containing

10% ethanol and lack of NAD-dependent glucose-6-phosphate dehydrogenase as well as genetic characteristics, illustrated that *Leuc. oenos* is distanced from but related to other *Leuconostoc* spp. Dicks et al. (1995) proposed the new genus *Oenococcus*, with *Oenococcus oeni* as the type species.

Using a polyphasic approach to the subdivision of the lactobacilli, Vandamme et al. (1996) proposed three phylogenetic groups that cut across the three traditional fermentation phenotypes of the lactobacilli. This includes new associations between the genera *Lactobacillus*, *Leuconostoc* and *Pediococcus*, as well as the newly described genera of *Weissella* and *Oenococcus* (Fig. 2). This illustrates the likely direction of future taxonomic developments in the bacteria.

### *5.5. Other genera closely related to the lactic acid bacteria*

A number of other gram-positive genera fit the broad definition of LAB but they are generally not considered to belong to this group (Vandamme et al., 1996). The genus *Aerococcus* forms tetrads and can be misidentified as coccus-shaped LAB resembling the pediococci (Weiss, 1991). Thus far it contained one species *A. viridans*. This organism has been known by the synonyms *Gaffkya homari* or *Pediococcus homari*; it is noted as the cause of a fatal disease of lobsters (Kelly and Evans, 1974) and has also been associated with human infection. *A. viridans* is closely related to the nonaciduric, microaerophilic *Pediococcus urinae-equi* which now have been separated from the pediococci and proposed as a second species for the genus *Aerococcus*. Subsequently, a third species *A. urinae* has been proposed (Aguirre and Collins, 1992). The name *Globicatella sanguis* has been applied to bacteria isolated from human clinical sources that have characteristics that place them among the LAB (Collins et al., 1992). With a G + C content of 37 mol% they are a genetically distinct group, with closest affinity to the *Aerococcus* clade. A new genus *Helcococcus* was established for the *Aerococcus*-like *Helcococcus kunzii* (Collins et al., 1993a).

Members of the genus *Gemella* grow as diplococci and comprise two species: *G. haemolysans* and *G. morbillorum* (Berger, 1991). They are commensals of the mucous membranes of humans and some warm-blooded animals. *G. haemolysans* was originally described as *Neisseria haemolysans* and *G. morbillorum* as *Diplococcus rubeolae* and later as *D. morbillorum*. After classification in the genus *Peptostreptococcus* and *Peptococcus* it was transferred to the genus *Streptococcus*. These bacteria are opportunistic pathogens, primarily of immunocompromised individuals. *G. morbillorum* is frequently found in the blood and throat of measles patients but its role in this disease seems to be auxiliary rather than causative. Measles is known as morbilli not rubella in both Germany and France. The genus *Dolosigranulum* contains one species, *D. pigrum* (Aguirre et al., 1993). The isolates were obtained from human clinical sources. They are characteristic of *Gemella* but they possess arginine dehydrolase. The genus is placed closer to *Aerococcus* than to other genera (Aguirre et al., 1993).

The causative agent of European foulbrood disease of the honey bee was assigned to the new genus *Melissococcus* as *M. pluton* (Bailey and Collins, 1982). Based on 16S rRNA gene sequence studies it was shown that this organism is closely related to the genus *Enterococcus* (Cai and Collins, 1994). The new genus *Lactosphaera* was described for the organism from anoxic digestor sludge, previously described as *Ruminococcus pasteurii* strain KoTa2 encompassing L-tartrate-fermenting anaerobic cocci, with *Lactosphaera pasteurii* KoTa2 as the type strain (Janssen et al., 1995). *L. pasteurii* produces considerable amounts of lactic acid, it is relatively aerotolerant and its 16S rDNA sequence suggests that it is closely related to the genus *Carnobacterium*, but it is distinguished by its coccus-shaped cells and its 45 mol% G + C content compared with 33–37 mol% of *Carnobacterium*.

The bifidobacteria were discovered in 1900 in the faeces of infants, but until the 1960s they received little attention from bacteriologists. Until 1957, in the 7th edition of Bergey's Manual of Determinative Bacteriology, they were included as *Lb. bifidus*, despite their recognition as a separate taxon by Orla-Jensen (1924). The bifidobacteria were included in the genus as *Lb. bifidus* until the

revision of Bergey's Manual of Systematic Bacteriology in 1986 when they were transferred to the genus *Bifidobacterium* (Scardovi, 1986). They are not true lactics because they produce lactic and acetic acids in the ratio of 2:3. The key enzyme in hexose fermentation that differentiates them from the lactics is a fructose-6-phosphate phosphoketolase that splits fructose phosphate to erythrose-4-phosphate and acetyl phosphate. A more important consideration for their exclusion from LAB is their high (55–57 mol%) G + C content in the DNA and their phylogenetic relatedness that places them in the *Actinomyces* subdivision of the gram-positive bacteria. They are important intestinal bacteria that are generally believed to contribute to desirable intestinal function and thereby to the well-being of the host (Mitsuoka, 1984). On account of such probiotic features *B. longum* (Biavati et al., 1991) and also *B. bifidum* and *B. infantis* have been incorporated into dairy products and therapeutic preparations for their health benefits. They may therefore constitute part of the viable microbial population of novel-type yoghurts, although their cultivation in milk substrate still appears to be a major technical obstacle. In the current version of Bergey's Manual (Sneath et al., 1986) a total of 24 species is listed and new species have been added up to a total of 29 presently (Biavati et al., 1991; Sgorbati et al., 1995). Marked morphological differences exist among species, and species differences have been confirmed by DNA–DNA homology and phenotypic techniques. The intestinal tract of humans and animals is the principal habitat for bifidobacteria, but they are also associated with sewage, human vaginal microflora, dental caries and the intestine of the honey bee and other insects. Their dominant association with the intestinal tract of infants (especially breast-fed) and newborns appears to be a key protective factor against enteric pathogens and as a stimulant of the immune system.

The genus *Brochothrix* comprises the catalase-positive bacteria that were originally assigned to the genus *Microbacterium* as a new species *M. thermosphactum* (McLean and Sulzbacher, 1953), despite marked differences from the type species of the genus, *Microbacterium lacticum*. The original description of *M. thermosphactum* indicated that it is heterofermentative (McLean and Sulzbacher, 1953) but this has not been confirmed. Later studies confirmed the differences between *M. lacticum* and *M. thermosphactum* and demonstrated that these organisms differ in cell morphology, enzyme profiles, peptidoglycan structure and DNA base composition. As a result, Sneath and Jones (1976) proposed the genus *Brochothrix* for the species *M. thermosphactum*. They are gram-positive, nonsporeforming, catalase-positive rods that have been isolated from a range of prepackaged meat and meat products (Jones, 1991). Talon et al. (1988) described a new species *B. campestris* that was isolated from soil and grass but differs from *B. thermosphacta* by several phenotypic characteristics. Original studies emphasised similarities between *Brochothrix* and *Lactobacillus*, so the genus was tentatively placed in the family *Lactobacillaceae* (Sneath and Jones, 1976), but subsequently it has been shown that *Brochothrix* more closely resembles *Listeria*. Both are catalase-positive and possess cytochromes and 16S rRNA oligonucleotide sequencing confirmed the close relationship between these bacteria (Sneath and Jones, 1986; Jones, 1991). *B. thermosphacta* is homofermentative producing L(+)-lactic acid from glucose, but under glucose-limitation, small amounts of other metabolites are detected (Grau, 1983). Spoilage of meat occurs when *B. thermosphacta* grows aerobically; it is not competitive under anaerobic conditions and it is rapidly outgrown by lactobacilli (especially *Lb. sake* and *Lb. curvatus*) in refrigerated vacuum-packaged meats and meat products. Under aerobic conditions *B. thermosphacta* produces acetoin and acetic acid from glucose (Blickstad and Molin, 1984) and short chain fatty acids from amino acids, resulting in sweet, sickly odours that spoil meat (Dainty et al., 1985). *B. thermosphacta* is a psychrotroph (growth limits 0 to 30°C), it does not grow at a pH of less than 5.0 and it is readily destroyed by heating at 63°C for 5 min.

The genus *Bacillus* forms part of the so-called *Clostridium* subdivision of gram-positive bacteria with a DNA base composition of less than 50 mol% G + C. Some phylogenetic relationship ex-

ists between *Sporolactobacillus* (represented by only *S. inulinus*) and *Bacillus*; this is also indicated by the presence of meso-diaminopimelic acid in their cell wall peptidoclycan. Two other aerobic to microaerophilic rod-shaped endospore-forming bacterial groups, *Amphibacillus* and *Alicyclobacillus*, have been recognised as distinct genera. *Alicyclobacillus acidocaldarius* and the thermophilic *A. acidoterererestris* are acidophilic and may cause flat sour spoilage of acidic beverages and fruit juices (Splittstoesser et al., 1994; Yamazaki et al., 1995). *Alicyclobacillus* spp. contain $\omega$-alicyclic fatty acids as major (and thus, far unique) fatty acid component (Yamazaki et al., 1996). The genus *Bacillus* does not comprise a uniform group of bacteria. *Sporolactobacillus* and *Amphibacillus* and also *B. coagulans*, *B. lentimorbis*, *B. popillae*, *B. smithii* and *B. stearothermophilus* differ from the 'typical' *Bacillus* species in their response to oxygen and their fermentation of glucose to lactic acid as the main end product. In addition, some strains do not produce catalase and lack a complete cytochrome system. *B. coagulans*, *B. smithii* and *B. stearothermophilus* are typically associated with the thermophilic, flat-sour spoilage of canned foods. Rainey et al. (1994) demonstrated the phylogenetic diversity of thermophilic *Bacillus* spp. using 16S rRNA analysis. The thermophilic, aerobic *Saccharococcus thermophilus* was isolated from sugar beet during extraction and described by Nystrand (1984). Subsequent studies place this organism close to the thermophilic group 5 bacilli that include *B. stearothermophilus* (Rainey and Stackebrandt, 1993).

## 6. Discussion and conclusions

Lactic acid bacteria are of major economic importance to the food industry. They predominate the natural microflora of many fermented foods where they serve a preservative or a spoilage role. They also play an important role in the digestive tract of humans and animals, especially in the young. With the appropriate environment, LAB dominate the natural microflora of milk, meats, vegetables and cereal products. The new processing technology of chill stored, modified atmosphere packaged foods has dramatically influenced the domination of LAB on meat and meat products. With the development of more sophisticated starter culture systems for an increasing number of foods and with the increasing interest in probiotics, it is important for food microbiologists to be current with taxonomic and nomenclatural changes among the LAB.

Over the years there has been a steady change in the taxonomy and nomenclature of bacteria, but in the past 10–20 years the changes have been dramatic. The original classification of bacteria was based on morphological characteristics. Sherman (1937) used morphological, physiological and serological characteristics to differentiate the streptococci. Unfortunately, growth conditions can markedly affect cell morphology; hence, LAB that were originally classified as lactobacilli have been reclassified, based on genetic data, e.g. *Lb. xylosus* (now *Lc.lactis* subsp. *lactis*) and *Lb. hordniae* (now *Lc. lactis* subsp. *hordniae*) (Schleifer et al., 1985). Molecular techniques allow a better understanding of the genetic relationships of bacteria; nonetheless, phenotypic characteristics such as carbohydrate fermentation patterns remain the benchmark of bacterial classification because they are the principal method available for the conventional microbiology laboratory.

Major changes in bacterial taxonomy started with the ability to determine the nucleotide ratios (G + C content) of bacterial DNA. With the exception of *Bifidobacterium* the bacteria traditionally considered as the LAB have a mol% G + C content below 55%. Although G + C content is not definitive, a broad range within a genus is a good indication that subdivision would be appropriate. The principles of genetic techniques available for taxonomic classification of the LAB were ably outlined by Pot et al. (1994). DNA:rRNA hybridisation applied to the streptococci led to the decision to establish *S. sensu stricto*, *Lactococcus* and *Enterococcus* as separate genera (Schleifer et al., 1985; Schleifer and Kilpper-Bälz, 1987).

Current techniques for studying phenotypic relationships are based on 16S and 23S rRNA sequence analysis. The genetic information contained in these molecules is highly conserved and

Table 11
Redefinition of the *Lactobacillus* clade of the lactic acid bacteria, crossing established generic and physiological distinctions, adapted from Vandamme et al. (1996)

| Phylogenetic group | Fermentation group 1 | Fermentation group 2 | Fermentation group 3 |
|---|---|---|---|
| | Obligate homofermenters | Facultative heterofermenters | Obligate heterofermenters |
| *Delbrueckii* group | *Lb. acidophilus*, *Lb. amylophilus*, *Lb. amylovorus*, *Lb. crispatus*, *Lb. delbrueckii* subsp. *delbrueckii*, *bulgaricus* and *lactis*, *Lb. gallinarum*, *Lb. gasseri*, *Lb. helveticus*, *Lb. jensenii*, *Lb. johnsonii*, *Lb. kefiranofaciens*, *Lb. kefirgranum* | *Lb. acetotolerans*, *Lb. hamsteri* | |
| *Lb. Casei-Pediococcus* group | *Lb. aviarius* subsp. *araffinosus* and *aviarius*, *Lb. farciminis*, *Lb. mali*, *Lb. ruminis*, *Lb. salivarius* subsp. *salicinus* and *salivarius*, *Lb. sharpae*, *Pediococcus damnosus*, *Pediococcus dextrinicum*, *Pediococcus parvulus* | *Lb. agilis*, *Lb. alimentarius*, *Lb. bifermentans*, *Lb. casei*, *Lb. coryniformis* subsp. *coryniformis* and *torquens*, *Lb. curvatus*, *Lb. graminis*, *Lb. homohiochii*, *Lb. intestinalis*, *Lb. murinus*, *Lb. paracasei* subsp. *paracasei* and *tolerans*, *Lb. pentosus*, *Lb. plantarum*, *Lb. paraplantarum*, *Lb. rhamnosus*, *Lb. sake* (*Lb. bavaricus*), *Pediococcus acidilactici* | *Pediococcus pentosaceus*, *Lb. brevis*, *Lb. buchneri*, *Lb. collinoides*, *Lb. fermentum*, *Lb. fructivorans*, *Lb. hilgardii*, *Lb. kefir*, *Lb. malefermentans*, *Lb. oris*, *Lb. parabuchneri*, *Lb. panis*, *Lb. parakefir*, *Lb. pontis*, *Lb. reuteri*, *Lb. sanfrancisco*, *Lb. suebicus*, *Lb. vaccinostercus*, *Lb. vaginalis* |
| *Leuconostoc* group | | | *Lb. fructosus*, *W. confusa* (*Lb. confusus*), *W. (Lb.) viridescens*, *W. (Lb.) halotolerans*, *W. (Lb.) hilgardii*, *W. (Lb.) kandleri*, *W. (Lb.) minor*, *W. hellenica*, *W. (Leuc.) paramesenteroides*, *Leuc. amelibiosum*, *Leuc. argentinum*, *Leuc. lactis*, *Leuc. mesenteroides*, *Leuc. pseudomesenteroides*, *Leuc. carnosum*, *Leuc. gelidum*, *Leuc. fallax* |

appropriate to determine the relatedness of bacteria. This has resulted in the proposal of dramatic changes in the taxonomy of the LAB; unfortunately, these new genetically-based taxonomic relationships cut across the phenotypic lines that have served food microbiologists well for many years. From a positive standpoint, the new divisions based on 16S and 23S rRNA sequence analysis used in association with other criteria (Vandamme et al., 1996) are likely to be definitive, but the reliability of the changes also depends on the appropriate selection of type strains to represent the species. A typical case are the proposals for reclassification of *Lb. casei* (Collins et al., 1989a; Dellaglio et al., 1991; Dicks et al., 1996). For the food microbiologist it is important that taxonomists carefully evaluate proposed changes, to avoid the necessity to reverse taxonomic changes.

Table 11, adapted from the comprehensive review by Vandamme et al. (1996), summarises the present status of the *Lactobacillus* clade. It is clear that these changes within the genus *Lactobacillus*, resulting (amongst others) from the 16S rRNA studies by Collins et al. (1991a), will have a dramatic effect on bacterial nomenclature for food microbiologists. Generic lines will not necessarily follow the convenient lines that developed from morphological and physiological characteristics and fermentation pathways. The establishment of a new genus *Weissella* (Collins et al., 1993b) encompassing the Paramesenteroides group including *Leuc. paramesenteroides* and some heterofermentative *Lactobacillus* species seems to be justified on a phylogenetic basis; this genus, however, now comprises morphologically different representatives of which *W. paramesenteroides* produces D(−) and the other DL-lactic acid as major end product of glucose fermentation. The eventual taxonomic decision with the *Lb. casei/Pediococcus* group might have a far greater impact on food microbiologists because this group includes the morphologically distinctive and homofermentative pediococci and more than 30 homofermentative as well as facultative and obligately heterofermentative *Lactobacillus* species. The genus *Carnobacterium* seems to be clearly established, but rules of bacterial nomenclature will dictate a change of *C. piscicola* to *C. maltaromicus* (Miller et al., 1974; Collins et al., 1991a). Similar changes will no doubt occur with other organisms as similarities are discovered with previously named species.

The genetic characterisation of the LAB is developing to the point where it is of increasing value to the manufacturer of starter cultures and to practical food microbiologists. DNA probes that can be used to differentiate *Lc. lactis* subsp. *cremoris* and *lactis* are already available (Klijn et al., 1991; Salama et al., 1991). Genetic probes are being developed that enable the specific identification of technically important LAB strains, even below the species level. The number of rRNA sequences that have been described for the LAB are considerable, and these numbers are increasing rapidly. This represents a valuable taxonomic tool that may ultimately be available to the routine food microbiologist.

## Acknowledgements

The authors gratefully acknowledge the support of the Canadian-German Cooperative Research Agreement Going Global Program (Canada) and the 'WTZ-Programm' (Germany) that facilitated this study.

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