

15 Taxonomy of Probiotic Microorganisms

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15.1 Introduction

When referring to probiotics, one refers to probiotic strains, i.e., the microbial individuals, sub-cultures of billion of almost identical cells ideally derived from the same mother cell. Therefore, beneficial effects attributed to probiotics are ascribed in fact to specific strains. However, these strains have to be, by law, clearly identified at the species level (Pineiro and Stanton, 2007). In fact, probiotics have to be safe for consumption, and the evaluation of QPS – qualified presumption of safety – status by the European Food Safety Authority (EFSA) (Opinion, 2007) is discussed for species, not for single strains.

Also, corrected names have to be reported on products labels: failure of identification of the declared species is a commercial fraud and a consumer misleading, besides being an indication of unreliability of the product.

These two examples should clarify how important is the correct taxonomic identification of probiotic strains in the assessment of their reliability and efficacy.

The aim of the present contribution is to clarify which procedures, rules and scientific knowledge stand behind microbial names, as results of taxonomic analysis. Probiotic strains described to date fall in two different groups of microorganisms, namely bacteria and yeasts, which will be the focus of this treatment.

15.2 What is Taxonomy?

Taxonomy, from the Greek meaning categorization, can be viewed as a quest for order in nature. In other words, it is the analysis of the existing biodiversity in a systematic way, with the aim of arranging it in an ordered hierarchical scheme. This hierarchy is (should be) the result of the genealogical relationships between organisms (the *taxa*). The information used for these purposes has to be as complete as possible, encompassing morphology, physiology, ecology and genetics.

A taxonomic procedure can be viewed as an iterative process, in which (i) organisms are clustered into groups on the basis of similarity; then (ii) groups are given formal names, indicated in italics, composed by a genus name and a specific identifier (e.g., *Homo sapiens*); and finally, other *taxa* are analyzed and can be either assigned to any already existing (i) and named (ii) group, or newly identified and described as novel groups. These three steps are indicated as *classification*, *nomenclature*, and *identification*, respectively (Staley and Krieg, 1989). The taxonomic hierarchy is based on the unit called species (see below). Then, Species are grouped in a Genus, Genera in a Family, Families in an Order, Orders in a Class, and Classes in a Domain. Three domains of life have been described comprising all the living organisms, two for the prokaryotes, Archaea and Bacteria, and one for eukaryotes. In the Domain Eukarya, four kingdoms are also recognized, i.e., Protista, Fungi, Animalia, and Plantae, to account for the diversity of eukaryotes, while no Kingdom category has been proposed above Classes in the Domains Archaea and Bacteria (Woese et al., 1990).

It is clear that a species in the bacterial domain must be, as a category, equivalent to a eukaryotic species, otherwise the “scheme of life” loses its meaning. However, clearly, biologically, species such as *Lactobacillus casei* (a bacterium) and ourselves as *Homo sapiens* are not comparable. Thus, it is clear that taxonomy is a necessary but conventional way of describing diversity. However, taxonomy has the important practical aim of making biodiversity accessible through cataloguing, therefore the species is considered to be equivalent as a category in the three domains, but it is circumscribed differently when analyzing different organisms.

15.2.1 Concept, Delineation and Naming of Species

The basic unit of the taxonomic scheme is the species. The species problem is one of the most debated topics in science, and will not be reviewed here: probiotic microorganisms are actually strains, not species, therefore it seems much more interesting here to focus on the relationship between species and strain, and explain how a species is circumscribed, i.e., which are the criteria used to determine if an isolate belongs to a known species or deserves a novel name.

Excellent reviews on the species concept and species circumscription are available for the two domains which include the probiotic strains known to date, i.e., Bacteria and Eukarya (Kingdom Fungi), (Giraud et al., 2008;

Rosselló-Mora and Amann, 2001). To date no archaeal strains with probiotic properties have been described, therefore that domain will not be reviewed here.

Considering species delineation, the practical criteria for the description of novel species will be reminded here. As for bacteria, the gene sequence for 16S rRNA gene is determined and analyzed to obtain the phylogenetic placement for the microorganism, then the closest neighbors are considered in a comparative study: DNA-DNA hybridization technique is then applied to assess the overall genomic similarity of the strains; if it is above 70% the strains are considered to belong to the same species, otherwise they represent different *taxa*. Also, relevant phenotypic characteristics of the strains are determined, e.g., patterns of fermentation of carbohydrates, in order to define a diagnostic trait which makes the novel species recognizable from its neighbors. More recently, other techniques have been suggested which should be useful in a more precise species delineation, such as Multi Locus Sequence Typing (Stackebrandt et al., 2002).

For fungi, and yeasts in particular, the procedure is less straightforward, and a novel species is described when genetic and/or morphological and/or reproductive differences are found, but these criteria can vary depending on the organisms under study.

This makes it clear that there are no rules for classification, as only experts can evaluate if a group of strains deserves a species status, and the standard procedure for species delineation of bacteria is pragmatic and useful, but does not have to be applied strictly.

On the contrary, rules do exist in nomenclature: once a group of strains is believed to represent a novel species, it deserves a name, which has to be given following precise regulation. Nomenclature of bacteria follows the International Code of Nomenclature of Bacteria (Lapage et al., 1990), and names are listed in the “Approved List 1980” and listed as Approved Lists 1980. Names published after 1980 or changes in names, to be valid, have to be published on the International Journal of Systematic and Evolutionary Microbiology – IJSEM – (known as International Journal of Systematic Bacteriology – IJSB – before 2000). They can also be published elsewhere, but, to be officially recognized, they have to be announced in the Validation Lists on IJSEM.

Nomenclature of yeasts follows, as for fungi, the International Code of Botanical Nomenclature, the recent version of which is the so-called Vienna Code, adopted by the Seventeenth International Botanical Congress, Vienna, Austria, July 2005 (McNeill et al., 2006). Species descriptions of unicellular eukaryotes are published on IJSEM, but also on other journals, and names are listed in the Index fungorum (see below for details).

As taxonomy is always linked to technical progress, taxonomic re-examinations often results in changes in nomenclature. Rules in nomenclature are necessary to avoid ambiguities and misunderstandings when taxonomic re-evaluations are performed and changes in names occur. The availability of lists of names, with rules of priority, also allows associating to new names characteristics linked to older ones, avoiding loss of information due to changes in names.

One of the most important points of the rules of nomenclature is that, when a novel species is described, a type strain (i.e., a strain chosen as a reference point among those grouped in the new species) has to be indicated, which has to be deposited in international culture collections, and be available to the scientific community for study and comparisons. Finally, it has to be pointed out that for bacteria one single correct name exists for a described species, while for yeasts there are two names, indicating the two different living forms, i.e., the teleomorph – the sexual reproductive stage, or the anamorph, the asexual reproductive stage.

15.2.3 Useful Links for Taxonomic Information

As anticipated, nomenclature is clearly regulated, and electronic versions of the Codes are available: the Bacteriological Code (Lapage et al., 1992) at <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=icnb.TOC&depth=2>, and the Vienna Code (McNeill et al., 2006) at <http://ibot.sav.sk/icbn/main.htm>.

Also, lists of up-to-date names can be checked online, on the DSMZ (German collection of microorganisms and cell cultures, <http://www.dsmz.de/bactnom/bactname.htm>) or on the internet pages compiled by J. P. Euzéby and updated after each issue of IJSEM (<http://www.bacterio.cict.fr/>). For yeasts the reference is the Index Fungorum, <http://www.indexfungorum.org/>.

For both bacteria and unicellular microorganisms, the journal publishing novel species descriptions is the International Journal of Systematic and Evolutionary Microbiology (www.sgmjournals.org).

Considering classification, the reference book for bacteria is the Bergey's Manual of Systematic Bacteriology, now at its second edition (2001), while, for yeasts, useful indications can be found in *The Yeasts: a Taxonomic Study*, fourth edition (1999) (Edited by Kurtzman and Fell), and in *Yeasts: Characteristics and Identification*, third edition (2000) (Cambridge: Cambridge University Press). These books provide information on morphologic, metabolic and genetic

characteristics of all known taxa and keys for their identification. Concerning prokaryotes, the taxonomic outline of the last version of Bergey's Manual underlines the phylogenetic placement of genera and taxonomic level above (Garrity et al., 2007a, <http://www.taxonomicoutline.org/>); a comprehensive picture of the phylogenetic diversity of prokaryotic microorganisms can be visualized at http://www.arb-silva.de/fileadmin/silva_databases/living_tree/LTP_tree_s93.pdf, which can also be used to analyze specific subtrees including the species of interest (Yarza et al., 2008).

Novel species descriptions require the deposit of the type strain in at least two recognized culture collections, the only international organizations devoted to storage and preservation of biodiversity. Well known collections are, among others, ATCC (www.atcc.org), LMG (www.bespo.be/bccm), DSMZ (www.dsmz.de) and JCM (<http://www.jcm.riken.jp/>), while CBS (<http://www.cbs.knaw.nl/>), DBVPG (<http://www.agr.unipg.it/dbvpg/>), and NRRL (<http://nrrl.ncaur.usda.gov/>) are mostly devoted to yeasts. Other information can be found on the web pages of the World Federation for Culture Collections (<http://www.wfcc.info/datacenter.html>), for other culture collections and abbreviations.

Finally, genome sequence data are expected to significantly contribute to taxonomy, therefore an interesting genomic database is GOLD (www.genomesonline.org), where sequencing projects are identified with a label: "Gc number" for complete genomes ([http://genomesonline.org/gold.cgi?want = Published + Complete + Genomes](http://genomesonline.org/gold.cgi?want=Published+Complete+Genomes)), and a "Gi number" for incomplete sequences ([http://www.genomesonline.org/gold.cgi?want = Bacterial + Ongoing + Genomes](http://www.genomesonline.org/gold.cgi?want=Bacterial+Ongoing+Genomes) and <http://www.genomesonline.org/gold.cgi?want=Eukaryotic+Ongoing+Genomes>, for Eukaryotes, respectively), which will be referred to in the text.

15.3 Taxonomic Placement of Probiotic Microorganisms

Members of the Domain Bacteria are unicellular prokaryotic microorganisms, characterized by different cellular shapes (cocci, rods, spiral etc.) and cell size of few micrometers. To date, more than 8,000 species of prokaryotes (Garrity et al., 2007a) are described, grouping organisms isolated from almost every habitat on earth.

Concerning the taxonomy of the Domain, it is subdivided into 24 Phyla. Gram-positive probiotic strains known to date belong to the Phyla *Firmicutes* and

Actinobacteria, with low or high genome guanine-cytosine base (GC) content, respectively. Also, probiotic properties have been attributed to *Escherichia coli*, a Gram-negative bacterium belonging to the Phylum *Proteobacteria*.

Data will be presented as follows: after some general indications concerning the bacterial and yeast genera involved, detailed information will be given on species comprising probiotic strains. Also, an updated list of species included in the respective genera will be presented, with the double aim of providing an immediate check list for correct names of strains and a reference to the original description of the species themselves. Species belonging to different genera will be treated separately, even if closely related (e.g., *Lactobacillus* and *Pediococcus*), to facilitate the consultation of data for single species and genera. Finally, all genus names will be reported *in extenso* throughout the text, to avoid confusion between names of different genera with the same capital letter (e.g., *B.* could stand for *Bifidobacterium*, *Bacillus* and *Brevibacillus*).

15.3.1 Lactic Acid Bacteria (LAB)

This group of bacteria is named after the ability of fermenting carbohydrate to lactic acid. Taxonomically, it comprises diverse genera of bacteria, which appear to be also phylogenetically unrelated. The two most important genera in the probiotic field are *Lactobacillus* and *Bifidobacterium*, but some others contain species of interest, e.g., *Pediococcus*, *Enterococcus*, and *Lactococcus*.

15.3.1.1 Genus *Lactobacillus*

Lactobacilli are Gram-positive bacteria, unable to sporulate, occurring as rods or cocco-bacilli, with a GC composition of the genome usually below 50% (low GC bacteria). They are fastidious microorganisms, requiring rich media to grow, and microaerophilic. They are catalase negative, even if pseudocatalase activity can sometimes be present in some strains and in presence of a heme group.

They are almost ubiquitous and can be found in almost all the environments where carbohydrates are available, such as food (dairy products, fermented meat, sourdoughs, vegetables, fruits, beverages), respiratory, gastro-intestinal (GI) and genital tracts of humans and animals, sewage and plant material.

The genus *Lactobacillus* belongs to the Phylum *Firmicutes*, Class *Bacilli*, Order *Lactobacillales*, Family *Lactobacillaceae* and its closest relatives, being

grouped within the same Family, are the genera *Paralactobacillus* and *Pediococcus* (Garrity et al., 2007b).

At the time of writing (September 2008), the genus includes 116 species with valid names, and several others are in course of publication (▶ [Table 15.1](#)).

The taxonomy of the genus is largely unsatisfactory, as the description of many novel species in the recent years has made it clear that the genus is heterogeneous and it is phylogenetically intermixed with the other two genera of the Family, i.e., *Pediococcus* and *Paralactobacillus* (▶ [Figure 15.1](#)). Moreover, metabolic differences, in terms of type and quantity of metabolites of carbohydrate fermentation, do not match with phylogenetic groupings and are, therefore, unreliable markers for their classification. Two species of the genus, namely *Lactobacillus catenaformis* and *Lactobacillus vitulinus* are only poorly related to the other species and their taxonomic status requires attention (Hammes and Vogel, 1995; Pot et al., 1994); for this reason they were omitted in ▶ [Figure 15.1](#).

Phylogenetically, the species form a number of stable groups, at least seven, based on the analysis of 16S rRNA gene sequences, but several species form distinct lines of descent (Felis and Dellaglio, 2007) as can be noted in the ▶ [Figure 15.1](#), depicting the updated structure of the taxonomic Family.

Lactobacillus Acidophilus

The etymology of the name, meaning acid-loving, indicates the preference of this species for acid medium for growth. Strains of this species usually display a rod shape with rounded ends and occur singly, in pairs and in short chains.

The original description was based on strains isolated from the intestinal tract of man and animals, human mouth and vagina, but are also easily found in milk and dairy products. At the time of first description, it was a heterogeneous species, as it included strains later reclassified as novel species, e.g., *Lactobacillus johnsonii*. Its metabolism is homofermentative, converting hexoses almost completely to lactic acid (both isomers D and L are produced) (Hammes and Vogel, 1995). Phylogenetically, it is placed in the *Lactobacillus delbrueckii* group (Felis and Dellaglio, 2007). The type strain is ATCC 4356^T (=LMG 9433^T = DSM 20079^T), of human origin, active for acidophilus milk when used with yeast extract. The GC content of the genome is 34–37%.

Genome sequence data are available for the non-type probiotic strain NCFM (strain Gc00252), and another sequencing project is ongoing (Gi02787), but there is no accession number indicating which particular strain is being sequenced.

■ Table 15.1

List of valid names in the genus *Lactobacillus* (updated at September 2008) (Cont'd p. 599)

	Species of the genus <i>Lactobacillus</i>
1	<i>Lactobacillus acetotolerans</i>
2	<i>Lactobacillus acidifarinae</i>
3	<i>Lactobacillus acidipiscis</i>
4	<u>Lactobacillus acidophilus</u>
5	<i>Lactobacillus agilis</i>
6	<i>Lactobacillus algidus</i>
7	<u>Lactobacillus alimentarius</u>
8	<u>Lactobacillus amylolyticus</u>
9	<i>Lactobacillus amylophilus</i>
10	<i>Lactobacillus amylotrophicus</i>
11	<u>Lactobacillus amylovorus</u>
12	<i>Lactobacillus animalis</i>
13	<i>Lactobacillus antri</i>
14	<i>Lactobacillus apodemi</i>
15	<u>Lactobacillus aviarius</u>
	<i>Lactobacillus aviarius</i> subsp. <i>araffinosus</i>
	<i>Lactobacillus aviarius</i> subsp. <i>aviarius</i>
16	<i>Lactobacillus bifermentans</i>
17	<u>Lactobacillus brevis</u>
18	<u>Lactobacillus buchneri</u>
19	<i>Lactobacillus camelliae</i>
20	<u>Lactobacillus casei</u>
21	<i>Lactobacillus catenaformis</i>
22	<i>Lactobacillus ceti</i>
23	<i>Lactobacillus coleohominis</i>
24	<i>Lactobacillus collinoides</i>
25	<i>Lactobacillus composti</i>
26	<i>Lactobacillus concavus</i>
27	<i>Lactobacillus coryniformis</i>
	<i>Lactobacillus coryniformis</i> subsp. <i>coryniformis</i>
	<i>Lactobacillus coryniformis</i> subsp. <i>torquens</i>
28	<u>Lactobacillus crispatus</u>
29	<i>Lactobacillus crustorum</i>
30	<u>Lactobacillus curvatus</u>
31	<u>Lactobacillus delbrueckii</u>

■ **Table 15.1** (Cont'd p. 600)

	Species of the genus <i>Lactobacillus</i>
	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>
	<i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i>
	<i>Lactobacillus delbrueckii</i> subsp. <i>indicus</i>
	<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>
32	<i>Lactobacillus diolivorans</i>
33	<i>Lactobacillus equi</i>
34	<i>Lactobacillus equigenerosi</i>
35	<u><i>Lactobacillus farciminis</i></u>
36	<i>Lactobacillus farraginis</i>
37	<u><i>Lactobacillus fermentum</i></u>
38	<i>Lactobacillus fornicalis</i>
39	<i>Lactobacillus fructivorans</i>
40	<i>Lactobacillus frumenti</i>
41	<i>Lactobacillus fuchuensis</i>
42	<u><i>Lactobacillus gallinarum</i></u>
43	<u><i>Lactobacillus gasseri</i></u>
44	<i>Lactobacillus gastricus</i>
45	<i>Lactobacillus ghanensis</i>
46	<i>Lactobacillus graminis</i>
47	<i>Lactobacillus hammesii</i>
48	<i>Lactobacillus hamsteri</i>
49	<i>Lactobacillus harbinensis</i>
50	<i>Lactobacillus hayakitensis</i>
51	<u><i>Lactobacillus helveticus</i></u>
52	<u><i>Lactobacillus hilgardii</i></u>
53	<i>Lactobacillus homohiochii</i>
54	<i>Lactobacillus iners</i>
55	<i>Lactobacillus ingluviei</i>
56	<i>Lactobacillus intestinalis</i>
57	<i>Lactobacillus jensenii</i>
58	<u><i>Lactobacillus johnsonii</i></u>
59	<i>Lactobacillus kalixensis</i>
60	<u><i>Lactobacillus kefiranofaciens</i></u>
	<i>Lactobacillus kefiranofaciens</i> subsp. <i>kefiranofaciens</i>
	<i>Lactobacillus kefiranofaciens</i> subsp. <i>kefirgranum</i>
61	<u><i>Lactobacillus kefiri</i></u>
62	<i>Lactobacillus kimchii</i>

■ **Table 15.1** (Cont'd p. 601)

	Species of the genus <i>Lactobacillus</i>
63	<i>Lactobacillus kitasatonis</i>
64	<i>Lactobacillus kunkeei</i>
65	<i>Lactobacillus lindneri</i>
66	<i>Lactobacillus malefermentans</i>
67	<i>Lactobacillus mali</i>
68	<i>Lactobacillus manihotivorans</i>
69	<i>Lactobacillus mindensis</i>
70	<u><i>Lactobacillus mucosae</i></u>
71	<i>Lactobacillus murinus</i>
72	<i>Lactobacillus nagelii</i>
73	<i>Lactobacillus namurensis</i>
74	<i>Lactobacillus nantensis</i>
75	<i>Lactobacillus oligofermentans</i>
76	<i>Lactobacillus oris</i>
77	<u><i>Lactobacillus panis</i></u>
78	<i>Lactobacillus pantheris</i>
79	<i>Lactobacillus parabrevis</i>
80	<i>Lactobacillus parabuchneri</i>
81	<u><i>Lactobacillus paracasei</i></u>
	<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i>
	<i>Lactobacillus paracasei</i> subsp. <i>tolerans</i>
82	<i>Lactobacillus paracollinoides</i>
83	<i>Lactobacillus parafarraginis</i>
84	<i>Lactobacillus parakefiri</i>
85	<i>Lactobacillus paralimentarius</i>
86	<u><i>Lactobacillus paraplantarum</i></u>
87	<u><i>Lactobacillus pentosus</i></u>
88	<i>Lactobacillus perolens</i>
89	<u><i>Lactobacillus plantarum</i></u>
	<i>Lactobacillus plantarum</i> subsp. <i>argenteratensis</i>
	<i>Lactobacillus plantarum</i> subsp. <i>plantarum</i>
90	<u><i>Lactobacillus pontis</i></u>
91	<i>Lactobacillus psittaci</i>
92	<i>Lactobacillus rennini</i>
93	<u><i>Lactobacillus reuteri</i></u>
94	<u><i>Lactobacillus rhamnosus</i></u>

■ Table 15.1

	Species of the genus <i>Lactobacillus</i>
95	<i>Lactobacillus rogosae</i>
96	<i>Lactobacillus rossiae</i>
97	<i>Lactobacillus ruminis</i>
98	<i>Lactobacillus saerimneri</i>
99	<u><i>Lactobacillus sakei</i></u>
	<i>Lactobacillus sakei</i> subsp. <i>carneus</i>
	<i>Lactobacillus sakei</i> subsp. <i>sakei</i>
100	<u><i>Lactobacillus salivarius</i></u>
101	<u><i>Lactobacillus sanfranciscensis</i></u>
102	<i>Lactobacillus satsumensis</i>
103	<i>Lactobacillus secaliphilus</i>
104	<i>Lactobacillus senmaizukei</i>
105	<i>Lactobacillus sharpeae</i>
106	<i>Lactobacillus siliginis</i>
107	<i>Lactobacillus spicheri</i>
108	<i>Lactobacillus suebicus</i>
109	<i>Lactobacillus thailandensis</i>
110	<i>Lactobacillus ultunensis</i>
111	<i>Lactobacillus vaccinostercus</i>
112	<i>Lactobacillus vaginalis</i>
113	<i>Lactobacillus versmoldensis</i>
114	<i>Lactobacillus vini</i>
115	<i>Lactobacillus vitulinus</i>
116	<i>Lactobacillus zymae</i>

Species with QPS status are underlined, principal species including probiotic strains are in bold and are described in the text

Lactobacillus Casei – Lactobacillus Paracasei

The two species *Lactobacillus casei* and *Lactobacillus paracasei* are presented together because they are closely related and their nomenclatural status has been discussed for a long time and only recently solved (Judicial Commission, 2008).

The name *casei* indicate the origin of the species in cheese and dairy products in general, but it has been isolated also from sourdough, silage, human GI tract (GIT), vagina, sewage etc. The name *paracasei* indicate the close resemblance of this *taxon* to *Lactobacillus casei*.

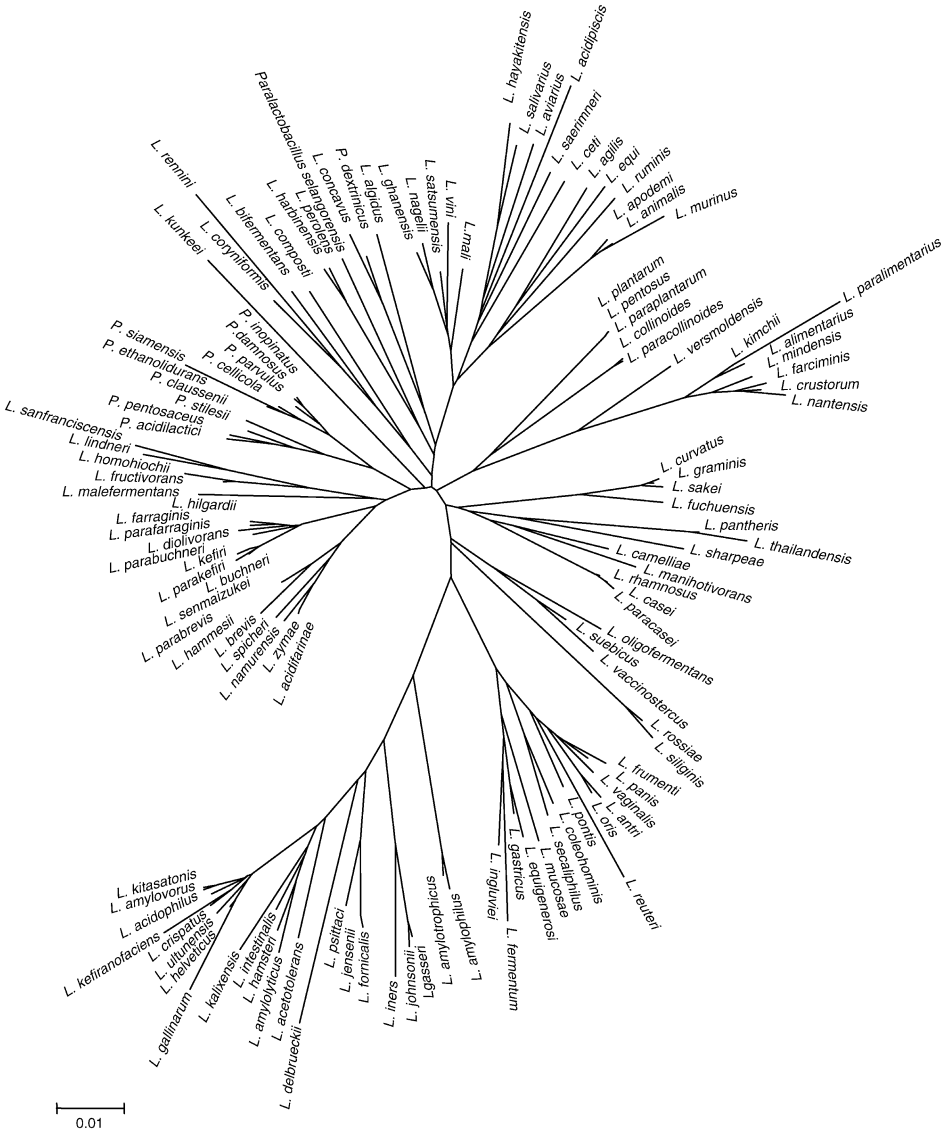
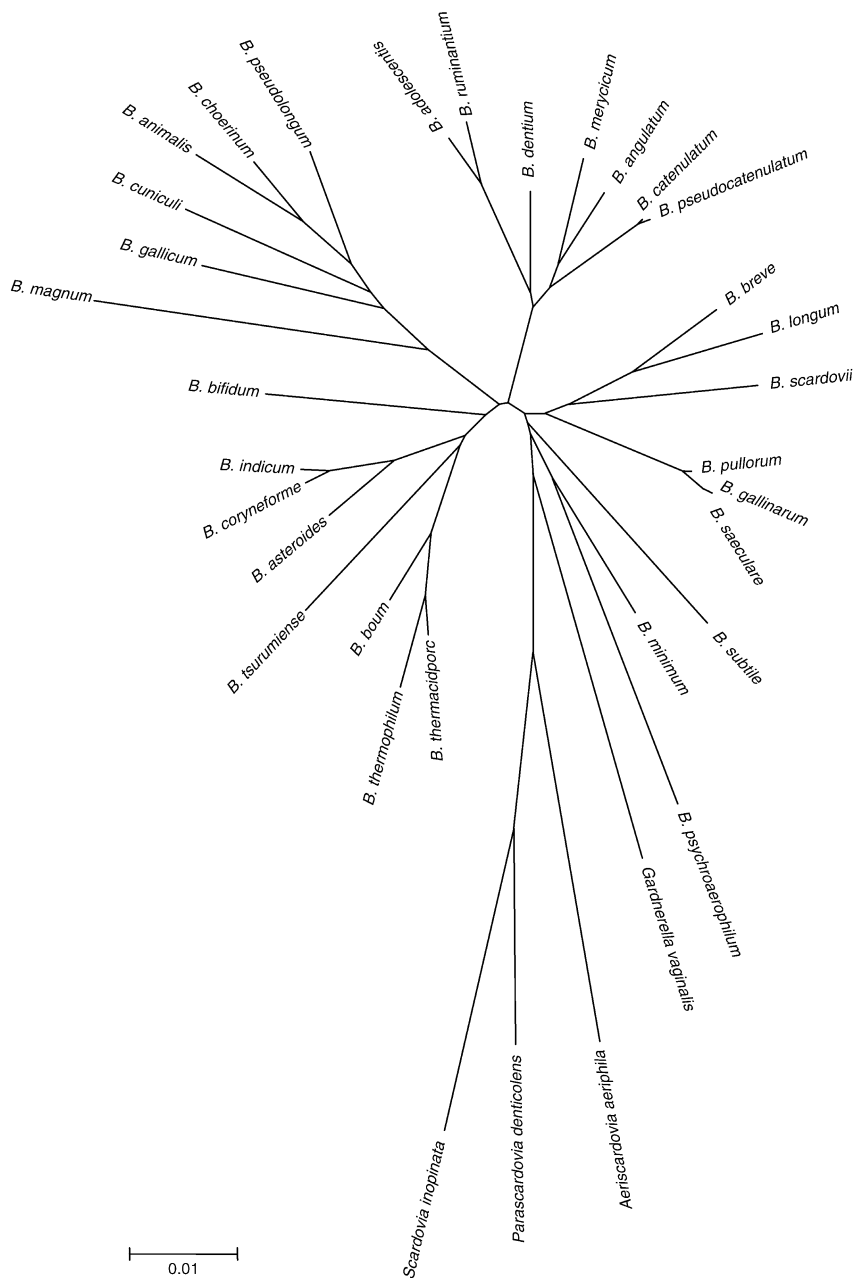


Figure 15.1

Phylogenetic tree showing the relationships among the species of the Family *Lactobacillaceae*, including genera *Lactobacillus* (abbreviated with "L." in the tree), *Paralactobacillus* and *Pediococcus* (abbreviated with "P." in the tree). The tree was calculated with a maximum-likelihood-derived distance method and clustering was performed with neighbor joining method. The bar indicates number of substitutions per site.



■ **Figure 15.2**

Phylogenetic tree depicting relationships among the species of the genus *Bifidobacterium* (abbreviated as "B." in the tree) and related genera. Tree was calculated with the same procedure reported for [Figure 15.1](#).

The first description of these rods with square ends, often occurring in chain, was based on few phenotypic traits, and the choice of strain ATCC 393 as the type has been considered inadequate for a long time. This has led to the description of *Lactobacillus paracasei*, phenotypically very similar but genomically distinct. The details of the taxonomic controversy will not be explained here, but it is important to point out that the debate has been definitively concluded as follows: both species names are valid, with ATCC 393^T as the type strain of *Lactobacillus casei* and NCFB 151^T (=ATCC 25302^T) as type strain of *Lactobacillus paracasei* (Judicial Commission, 2008). This is expected to have important consequences also in marketing of probiotic strains: many of the cultures on the market called *Lactobacillus casei* are actually more similar to the type strain of *Lactobacillus paracasei* and therefore should be given that name.

As for metabolism, both species are facultatively heterofermentative microorganisms, producing lactic acid (L or both isomers, depending on the strains) from hexoses, but are also able to ferment pentoses (Hammes and Vogel, 1995). Phylogenetically, the two species together with *Lactobacillus rhamnosus* form a distinct clade in the genus *Lactobacillus* (Felis and Dellaglio, 2007), and the genome GC content for both species ranges between 45 and 47%.

Genome data have been obtained or are in progress for several strains in the two species (Gc00438, Gc00822, Gi02995, Gi03110, Gi02219, and Gi02799), including the type strain of *Lactobacillus casei* ATCC 393^T. However, the identification of the strains is not always clear, e.g., ATCC 334 (Gc00438) is indicated as *Lactobacillus casei* while it is a *Lactobacillus paracasei* strain (Judicial Commission, 2008).

Lactobacillus Crispatus

The curled morphology of the cells in liquid medium gives the name to the species, of straight or slightly curved rods, occurring singly or in short chains. Phylogenetically related to *Lactobacillus acidophilus* and *Lactobacillus delbrueckii*, its neighbor species appear to be *Lactobacillus kefiranofaciens*. Historically, the species was described based on strains previously assigned to the species *Lactobacillus acidophilus*, which showed a similar homofermentative metabolism, and also similar genome GC content (35–38% for *Lactobacillus crispatus*). Strains have been isolated from saliva, feces and urogenital tract of man and chicken, and also found in patients with different infections, but not in a causative role. The type strain is ATCC 33820^T (=LMG 9479^T = DSM 20584^T), isolated from human specimen (eye). Genome projects involving six non-type strains are ongoing (Gi02791, Gi02792, Gi03435, Gi03436, Gi03437, and Gi03438).

Lactobacillus Delbrueckii subsp. Bulgaricus

Lactobacillus delbrueckii is the type species of the genus *Lactobacillus*, and it currently includes four subspecies (☛ Table 15.1), three of which, i.e., subsp. *delbrueckii*, *bulgaricus*, and *lactis*, were first described as separate species. The unification under the same name then was performed, the name being *Lactobacillus delbrueckii*, as a rule of priority as it was described before the others. The species name is to honor Delbrück, a German bacteriologist, while *bulgaricus* indicate the geographical origin of the first isolated strains, Bulgaria. The evolution of strains of the subsp. *bulgaricus* has probably been driven by mankind: it has been used for centuries in the production of yogurt and of other dairy products; therefore its genetic make up is devoted to fermentation in milk at high temperature (Germond et al., 2003). This could also explain the unusually high genome GC content for the species, 49–51%, and the scarce ability to ferment sugars other than lactose. Also, genome reduction has been observed (van de Guchte et al., 2006), probably motivated by the proto-cooperative interaction with *Streptococcus thermophilus*, the partner for yogurt production.

Phylogenetically, the species *Lactobacillus delbrueckii* belongs to the homonymous clade in the genus *Lactobacillus*, which includes, besides the already mentioned *Lactobacillus acidophilus* and *Lactobacillus crispatus*, *Lactobacillus acetotolerans*, *Lactobacillus amylolyticus*, *Lactobacillus amylophilus*, *Lactobacillus amylophobicus*, *Lactobacillus amylovorus*, *Lactobacillus fornicalis*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, *Lactobacillus hamsteri*, *Lactobacillus helveticus*, *Lactobacillus iners*, *Lactobacillus intestinalis*, *Lactobacillus jensenii*, *Lactobacillus johnsonii*, *Lactobacillus kalixensis*, *Lactobacillus kefirifaciens*, *Lactobacillus kitasatonis*, *Lactobacillus psittaci*, and *Lactobacillus ultunensis*.

The type strain of the species *Lactobacillus delbrueckii* corresponds to the type strain of the homonymous subspecies and is ATCC 9649^T (=DSM 20074^T = LMG 6412^T), isolated from distillery sour grain mash incubated at 45°C, while the type strain of subsp. *bulgaricus* is ATCC 11842^T (=DSM 20081^T = LMG 6901^T), isolated from Bulgarian yogurt. Genome data are available for three strains of the species (Gc00443, Gc00394, and Gi02793), including also the type strain (Gc00394).

Lactobacillus Johnsonii

Another bacteriologist, Johnson, gives his name to the species *Lactobacillus johnsonii*, which shares with *Lactobacillus delbrueckii* not only the origin of the name, but also the phylogenetic placement and the homofermentative metabolism. Strains of this species were initially identified as *Lactobacillus acidophilus*

and *Lactobacillus crispatus*: with those two species *Lactobacillus johnsonii* shares different traits, such as similar GC content (33–35% in this case), the isomer of lactic acid produced (DL) and the homofermentative metabolism. Strains of *Lactobacillus johnsonii* have been isolated from human specimens but also from feces of animals. Type strain is ATCC 33200^T (=DSM 10533^T = LMG9436^T) and genome data available at the moment are for a probiotic strain NCC533 (Gc00171), while another is in progress, but no indication of the strain is available to date (Gi02798).

Lactobacillus Plantarum

The species *Lactobacillus plantarum* is one of the first taxa described in the genus. As the name indicates, it is commonly found in plant material, but it is also commonly found in a variety of environments, i.e., dairy products, human specimens, sewage etc. This flexibility determines also the genetic and phenotypic heterogeneity of the species, in which a novel subspecies has been recently delineated (► [Table 15.1](#)). Anyway, in general, the morphology of cells is rod-like with rounded ends, metabolism is facultatively heterofermentative, genome GC content is in the range of 44–46% and both isomers of lactic acid are produced from carbohydrates. From a phylogenetic standpoint, *Lactobacillus plantarum* closest relatives are *Lactobacillus pentosus* and *Lactobacillus paraplan-tarum*, and the three species form a distinct line of descent in the genus *Lactobacillus*.

The type strain is ATCC 14917^T (=DSM 20174^T = LMG 6907^T), isolated from pickled cabbage, and genome sequencing has been performed on a non-type strain with probiotic properties, WCFS1 (Gc00122), the first *Lactobacillus* strain sequenced (Kleerebezem et al., 2003), which is already becoming the model organism for the genus.

Lactobacillus Reuteri

The bent rods with rounded ends belonging to this species owe their name to the German microbiologist Reuter, who was the first to isolate and study these heterofermentative bacteria. Strains of *Lactobacillus reuteri* are isolated from intestine and feces of humans and animals, sourdough and meat. Often an antimicrobial compound, reuterin, is produced. *Lactobacillus reuteri* strains produce both isomers of lactic acid and have a genome GC content between 40 and 42%. Its closest phylogenetic relatives are *Lactobacillus antri*, *Lactobacillus coleohominis*, *Lactobacillus fermentum*, *Lactobacillus frumenti*, *Lactobacillus gastricus*, *Lactobacillus ingluviei*, *Lactobacillus mucosae*, *Lactobacillus*

oris, *Lactobacillus panis*, *Lactobacillus pontis*, *Lactobacillus secaliphilus*, and *Lactobacillus vaginalis*, which constitute the *Lactobacillus reuteri* subclade in the genus *Lactobacillus* (Felis and Dellaglio, 2007).

The type strain of the species, isolated from human intestine is ATCC 23272^T (=DSM 20016^T = LMG 9213^T), which genome has also been sequenced by two groups (Gc00573 and Gc00786), together with other four strains (Gi01862, Gi01863, Gi00762, and Gi02800).

Lactobacillus Rhamnosus

The ability to ferment rhamnose is one of the peculiarities of these rod-shaped bacteria, phylogenetically related to *Lactobacillus casei*, which can be isolated from dairy products, sewage, and human specimens. Strains of *Lactobacillus rhamnosus* have a facultatively heterofermentative metabolism, and are, in general very similar to *Lactobacillus paracasei* strains. Also, strains of this species are among the few in the genus *Lactobacillus* being assigned to Risk group 2 and are subjected to restricted distribution (<http://www.dsmz.de/microorganisms/html/bacteria.genus/lactobacillus.html>) as some strains have been isolated from endocarditis and other opportunistic infections. Nevertheless, the species has been included in list for QPS status and some strains are used as probiotics. Type strain of the species is ATCC 7469^T (=DSM 20021^T = LMG 6400^T) and three genome sequencing projects are ongoing (Gi02218, Gi00316, and Gi02801).

Lactobacillus Salivarius

As the name indicates, strains of *Lactobacillus salivarius* are common in human saliva, as well as in mouth and intestinal tract of man and animals. Historically considered a homofermentative species, after a recent taxonomic reevaluation (Lee et al., 2006), it is regarded as a facultatively heterofermentative species, able to ferment both hexoses and pentoses. Many recently described species have been found to be phylogenetically related to *Lactobacillus salivarius*, which clade is at present constituted by *Lactobacillus acidipiscis*, *Lactobacillus agilis*, *Lactobacillus animalis*, *Lactobacillus apodemi*, *Lactobacillus aviarius*, *Lactobacillus ceti*, *Lactobacillus equi*, *Lactobacillus ghanensis*, *Lactobacillus hayakitensis*, *Lactobacillus mali*, *Lactobacillus murinus*, *Lactobacillus nagelii*, *Lactobacillus ruminis*, *Lactobacillus saerimneri*, *Lactobacillus satsumensis*, and *Lactobacillus vini*.

Strains of this species produce L-lactic acid, and have a genome GC content of 34–36%. Type strain is ATCCC 11742^T (=DSM 20555^T = LMG 9477^T), isolated from human saliva, while the genome sequence has been obtained for a non-type strain with probiotic properties, UCC118 (Gc00362).

Interestingly, labels of some commercial products refer to the name “*Lactobacillus sporogenes*”: this name is invalid, does not indicate any *Lactobacillus* species, but the spore-forming *Bacillus coagulans* (Sanders et al., 2003) (see below).

15.3.1.2 Genus *Bifidobacterium*

The genus *Bifidobacterium*, even if traditionally listed among LAB, is only poorly phylogenetically related to genuine LAB: it belongs to the Phylum *Actinobacteria*, Class “*Actinobacteria*,” Order *Bifidobacteriales*, Family *Bifidobacteriaceae*, its neighbor genera being *Aeriscardovia*, *Gardnerella*, *Parascardovia*, and *Scardovia* (Garrity et al., 2007c). Species of these genera use a metabolic pathway for the degradation of hexoses, the so-called “bifid shunt,” different from that of *Lactobacillus* and related genera (Sgorbati et al., 1995; Ventura et al., 2004). The key enzyme, considered a taxonomic character for the identification of this group of bacteria, is fructose-6-phosphoketolase (EC 4.1.2.2). The genus includes, at present, 30 species (🔗 [Table 15.2](#)).

Bifidobacteria are Gram-positive rods, which can sometimes be branched, a characteristic which gives the name to the genus. Bifidobacteria do not form spores, are nonmotile, and anaerobic. Their genome GC content varies from 42 to 67 mol% and in fact they belong to the high GC Gram-positive bacteria. Five phylogenetic groups have been observed in the genus with also species constituting single lines of descent (Felis and Dellaglio, 2007; Matsuki et al., 2003).

Bifidobacterial strains exhibiting probiotic properties belong to the species *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium longum*, which are not related from a phylogenetic standpoint.

Bifidobacterium Adolescentis

Bacteria belonging to this species have been isolated from feces of human adult, bovine rumen and sewage. They are phylogenetically related with *Bifidobacterium angulatum*, *Bifidobacterium catenulatum*, *Bifidobacterium dentium*, *Bifidobacterium merycicum*, *Bifidobacterium pseudocatenulatum* and *Bifidobacterium ruminantium*, and their phenotypic differentiation from *Bifidobacterium dentium* is sometimes difficult. It is characterized by genome GC content of about 58%, while the cell-wall amino acids consist of Lys (Orn) – D-Asp. The type strain, isolated from human intestine is ATCC 15703^T (=DSM 20083^T = LMG 10502^T),

■ **Table 15.2**

List of valid names in the genus *Bifidobacterium* (updated at September 2008) (Cont'd p. 610)

	Species of the genus <i>Bifidobacterium</i>
1	<i>Bifidobacterium adolescentis</i>
2	<i>Bifidobacterium angulatum</i>
3	<i>Bifidobacterium animalis</i>
	<i>Bifidobacterium animalis</i> subsp. <i>animalis</i>
	<i>Bifidobacterium animalis</i> subsp. <i>Lactis</i>
4	<i>Bifidobacterium asteroides</i>
5	<i>Bifidobacterium bifidum</i>
6	<i>Bifidobacterium boum</i>
7	<i>Bifidobacterium breve</i>
8	<i>Bifidobacterium catenulatum</i>
9	<i>Bifidobacterium choerinum</i>
10	<i>Bifidobacterium coryneforme</i>
11	<i>Bifidobacterium cuniculi</i>
12	<i>Bifidobacterium dentium</i>
13	<i>Bifidobacterium gallicum</i>
14	<i>Bifidobacterium gallinarum</i>
15	<i>Bifidobacterium indicum</i>
16	<i>Bifidobacterium longum</i>
	<i>Bifidobacterium longum</i> subsp. <i>infantis</i>
	<i>Bifidobacterium longum</i> subsp. <i>longum</i>
	<i>Bifidobacterium longum</i> subsp. <i>Suis</i>
17	<i>Bifidobacterium magnum</i>
18	<i>Bifidobacterium merycicum</i>
19	<i>Bifidobacterium minimum</i>
20	<i>Bifidobacterium pseudocatenulatum</i>
21	<i>Bifidobacterium pseudolongum</i>
	<i>Bifidobacterium pseudolongum</i> subsp. <i>globosum</i>
	<i>Bifidobacterium pseudolongum</i> subsp. <i>Pseudolongum</i>
22	<i>Bifidobacterium psychraerophilum</i>
23	<i>Bifidobacterium pullorum</i>
24	<i>Bifidobacterium ruminantium</i>
25	<i>Bifidobacterium saeculare</i>
26	<i>Bifidobacterium scardovii</i>
27	<i>Bifidobacterium subtile</i>

■ Table 15.2

Species of the genus <i>Bifidobacterium</i>	
28	<u><i>Bifidobacterium thermacidophilum</i></u>
	<i>Bifidobacterium thermacidophilum</i> subsp. <i>Porcinum</i>
	<i>Bifidobacterium thermacidophilum</i> subsp. <i>thermacidophilum</i>
29	<u><i>Bifidobacterium thermophilum</i></u>
30	<u><i>Bifidobacterium tsurumiense</i></u>

Species with QPS status are underlined, principal species including probiotic strains are in bold and are described in the text

which genome sequence is also available (Gc00470); a second one is ongoing (Gi01706).

Bifidobacterium Animalis

The first description of this species was based on strains isolated from feces of different animals, but different data revealed that strains described as *Bifidobacterium lactis*, isolated from dairy products, belong to the same species, but to different subspecies (Masco et al., 2004). Strains of the subsp. *lactis* are more oxygen tolerant than those of the subsp. *animalis*, and this trait is very useful for probiotic application, as it allows them to survive in high number in the non-anaerobic conditions of commercial products; another difference between the two subspecies is the ability to grow in milk. *Bifidobacterium animalis* belongs to the *Bifidobacterium pseudolongum* phylogenetic group, which includes *Bifidobacterium choerinum*, *Bifidobacterium cuniculi*, *Bifidobacterium gallicum*, and, obviously, *Bifidobacterium pseudolongum*. The DNA G + C content of the species is 61%. The type strain of *Bifidobacterium animalis* subsp. *animalis* is ATCC 25527^T (=LMG 10508^T = DSM 20104^T), isolated from rat feces, type strain of *B. animalis* subsp. *lactis* is LMG 18314^T (=DSM 10140^T), isolated from fermented milk. Genome data for three strains will be available in the future for three non-type strains of also probiotic interest (Gi01876, Gi01988, and Gi03035).

Bifidobacterium Bifidum

Bifidobacterium bifidum is the type species of the genus, strictly anaerobic. It is among the most recognizable species in the genus on the basis of the fermentation pattern, and is also clearly different from other bifidobacteria phylogenetically. Cells have, in particular conditions, a peculiar “amphora-like” shape, and often appear also as branched rods (from which the name *bifidum*). Strains of this

species have been isolated from feces of humans, both adults and infants, human vagina and animal feces, but are readily used for production of fermented dairy products for probiotic purposes. The type strain is ATCC 29521^T (=DSM 20239^T = LMG 10645^T), isolated from feces of a breast-fed infant; the genome sequence for three strains, including two different subcultures of the type strain, are in progress (Gi02210, Gi02653, Gi02657).

Bifidobacterium Breve

Strains of this species are characterized by the short size, with or without bifurcation, with lysine and glycine as aminoacid in the murein, and have a genome GC content of about 58%. Strains of this species have been isolated from intestine of infants, human vagina, and sewage, and are phylogenetically related to *Bifidobacterium longum*. The type strain is ATCC 15700^T (=DSM 20213^T = LMG 11042^T) and three genome sequencing programs are ongoing, two for the different subcultures of the type strain and one for a non-type with probiotic properties (Gi00078, Gi02209, and Gi02654).

Bifidobacterium Longum

Differently from *Bifidobacterium breve*, cells of strains in this species show a very elongated cell shape with rare branching. It is anaerobic and considered the most common species of bifidobacteria, and is isolated from feces of infants and adults, human vagina, but also animal feces. Its closest phylogenetic relative is *Bifidobacterium breve*, and the two species form a couple quite unrelated to the other species in the bifidobacterial phylogenetic tree. Other relevant characteristic for strains in the species are the genome GC content of about 60%, and the presence of many plasmids, a unique feature among fecal bifidobacteria.

Two taxa formerly described as distinct species have been included in *B. longum*, namely *Bifidobacterium infantis* and *Bifidobacterium suis*. These bacteria, first recognized as biotypes of the species, have very recently been recognized as novel subspecies. The three subgroups have been isolated from slightly different niches, such as the GIT of human adults (subsp. *longum*), the GIT of infants (subsp. *infantis*), and pig's feces (subsp. *suis*).

The type strain of subsp. *longum* (and therefore of the species) is ATCC 15707^T (=DSM 20219^T = LMG 13197^T), isolated from feces of a human adult, for subsp. *infantis* is ATCC 15697^T (=DSM 20088^T = LMG 8811^T), isolated from feces of a human infant, and for subsp. *suis* is ATCC 27533^T (=DSM 20211^T = LMG 21814^T), isolated from feces of a piglet (Mattarelli et al., 2008).

Sequence data are already or will be soon available for six strains, including the type strain (Gc00811, Gc00108, Gi02659, Gi01539, and Gi02485).

15.3.1.3 Other LAB: Genera *Streptococcus*, *Lactococcus*, *Enterococcus*, and *Pediococcus*

Other species belonging to the true LAB group play an important role in food microbiology and nutrition and are, in some cases, also considered probiotics. Two taxa having an outstanding role are *Streptococcus thermophilus* and *Lactococcus lactis*. Strains of the former species are, in combination with *Lactobacillus delbrueckii* subsp. *bulgaricus*, the actors of yogurt production, while the latter is largely used for production of dairy products and, in recent years, has become the model organism for lactic acid bacteria. Other genera, which include strains attributed with probiotic properties are *Enterococcus* and *Pediococcus* and will be briefly treated below.

15.3.1.4 Genera *Streptococcus* and *Lactococcus*

The genera *Lactococcus* and *Streptococcus* form the Family *Streptococcaceae* of the Order *Lactobacillales* in the Class *Bacilli* of the Phylum *Firmicutes*.

The genus *Streptococcus* contains about 67 species of coccoid Gram positive bacteria, mostly known for the pathogenicity of some species. The species *Streptococcus thermophilus*, on the contrary, is known for its GRAS (generally recognized as safe status), due to the long history of use in food production. Also, genomic data have demonstrated that *Streptococcus thermophilus* has lost or inactivated the virulence-related genes characterized in pathogenic streptococci, during the adaptation to milk, confirming its safety (Delorme, 2008).

The genus *Lactococcus* groups coccoid bacteria formerly referred to as mesophilic lactic streptococci. It is constituted by six species and the most important for dairy fermentation is *Lactococcus lactis*, which is divided into three subspecies, namely *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *hordniae* and *Lactococcus lactis* subsp. *lactis*, which include the biovar *diacetylactis*. The diversity within the species has recently been re-evaluated with molecular analyzes, confirming that phenotypic and genotypic diversity are not coherent (Rademaker et al., 2007).

Streptococcus Thermophilus

As the name clearly indicates, strains of this *taxon* have a preference for growth at high temperature: all of them grow at 45°C, most are able to grow up to 50°C, and some survive also after heating 60°C for 30 min. This characteristic is an indication of man-driven adaptation to yogurt production, as already explained for *Lactobacillus delbrueckii* subsp. *bulgaricus*. In the case of *Streptococcus thermophilus*, however, genome GC content is in the range 37–40%, therefore no shift towards higher percentages is evident (Hardie and Whiley, 1995). A close phylogenetic relationship with *Streptococcus salivarius* has determined some nomenclatural changes in the past, with fluctuations of *Streptococcus thermophilus* between the status of species or of subspecies (“*Streptococcus salivarius* subsp. *thermophilus*”). However at present it is fully considered in the taxonomic rank of species, although some confusion is still present in literature data.

Streptococcus thermophilus has complex nutritional requirements, in particular considering aminoacids, which are most probably the result of the adaptation to growth in a rich medium such as milk, and also a consequence of proto-cooperation with *Lactobacillus delbrueckii* subsp. *bulgaricus*. The type strain is ATCC 19258^T (=DMS 20617 = LMG 6898), isolated from milk, and genome sequence data are available or in progress for four strains (Gc00451, Gc00234, Gc00233, and Gi00621).

Lactococcus Lactis

Lactococcus lactis is the type species of the genus *Lactococcus* (↪ Table 15.3) and, as both genus and species names suggest, it is strictly associated with milk. However,

■ Table 15.3

List of valid names in the genus *Lactococcus* (updated at September 2008)

Species of the genus <i>Lactococcus</i>	
1	<i>Lactococcus chungangensis</i>
2	<i>Lactococcus garvieae</i>
3	<u><i>Lactococcus lactis</i></u>
	<i>Lactococcus lactis</i> subsp. <i>cremoris</i>
	<i>Lactococcus lactis</i> subsp. <i>hordniae</i>
	<i>Lactococcus lactis</i> subsp. <i>lactis</i>
4	<i>Lactococcus piscium</i>
5	<i>Lactococcus plantarum</i>
6	<i>Lactococcus raffinolactis</i>

Lactococcus lactis, underlined, is the only species in the genus under consideration for QPS status

one of the three subspecies it includes has been described based on strains isolated from leafhopper (subsp. *hordniae*). Moreover, *Lactococcus lactis* is considered an “old” microorganism, originally colonizing plants, and only in recent time adapted to milk. Morphologically, cells are usually spherical or ovoid, in pairs or in chains, mesophilic with a homofermentative metabolism. Genome GC content is in the range 34–36%.

Nowadays, *Lactococcus lactis* is among the lactic acid bacterial species with the biggest economic importance, as strains are largely applied as starter cultures for a variety of products; it is the model species for the genetic and metabolic study of low GC Gram-positive bacteria, second only to *Bacillus subtilis* in importance. This is also causing some confusion in nomenclature of strains in the species: it has been anticipated above that genomic groups within the species are not phenotypically homogeneous. From a taxonomic standpoint, nomenclature of subspecies depends on the results of phenotypic tests (degradation of maltose, ribose, deamination of arginine, growth in presence of sodium chloride among others) (Teuber, 1995). However, the availability of genome data and the interest in genetic traits is prevailing over classical phenotypic studies: a clear example is that of strain MG1363 (Wegmann et al., 2007): it displays many of subsp. *lactis* phenotypic traits, but it is usually referred to as subsp. *cremoris*, due to its genetic similarity to the type strain of subsp. *cremoris*. The availability of the genome sequence and the use of the strain as an exemplary for the name *Lactococcus lactis* subsp. *cremoris*, therefore the genetic-based nomenclature will probably overcome the classical and phenotype-based correct one, generating a dichotomy in taxonomic procedure of identification of strains at the subspecies level.

Type strains of the three subspecies are ATCC 19435^T (=DSM 20481^T = LMG 6898^T) for subsp. *lactis* (and therefore for the whole species), ATCC 19257^T (=DSM 20069^T = LMG 6897^T) for subsp. *cremoris*, both isolated from milk and ATCC 29071^T (=DSM 20450^T = LMG 8520^T) for subsp. *hordniae*, isolated from the insect *Hordnia circellata*.

Three genome sequences are already available (Gc00054, Gc00450, and Gc508) and other will be available soon (Gi03379).

15.3.1.5 Genus *Enterococcus*

Related to the above described two genera, the genus *Enterococcus* is also important for the field of probiotics. The first bulk of species in the genus (*Enterococcus faecalis* and *Enterococcus faecium*) were previously described as streptococci, but

many others have followed, increasing the number of species in the genus to 35 (▶ [Table 15.4](#)).

Potential probiotic properties have been reported for strains of *Enterococcus faecium* but bacteria in the genus *Enterococcus* are amongst the leading causes of community- and nosocomial infections. Therefore the Scientific Committee of the European Authority for Food Safety (EFSA) did not propose QPS status for any species of genus *Enterococcus*.

From a taxonomic standpoint, the genus *Enterococcus* falls into the Family *Enterococcaceae*, together with *Atopobacter*, *Catelliococcus*, *Melissococcus*, *Pilibacter*, *Tetragenococcus*, and *Vagococcus*.

Enterococcus Faecium

As the name clearly indicates, source of first isolation of *Enterococcus faecium* was fecal material, but strains of this species are frequently isolated not only from the GIT of animals (mammals, birds, and reptiles), but also from raw milk and dairy products (Devriese and Pot, 1995).

Cells are ovoid, occurring in pairs and short chains, not pigmented and not motile. Genome GC content ranges from 37 to 40%. *Enterococcus faecium* gives a name also to a phylogenetic group of closely related species in the genus, which, after description of novel species (▶ [Table 15.4](#)), includes *Enterococcus durans*, *Enterococcus hirae*, *Enterococcus mundtii*, *Enterococcus ratti*, and *Enterococcus villorum*.

Type strain is ATCC 19434^T (=DSM 20477^T = LMG 11423^T), and genome sequence data are not available at the moment but are in progress for 11 strains (Gi00227, Gi00228, Gi00229, Gi02729, Gi03235, Gi03232, Gi03265, Gi03306, Gi03225, Gi02730, and Gi03362).

15.3.1.6 Genus *Pediococcus*

Another genus including coccoid bacteria, relevant for the area of probiotics, is *Pediococcus*. Its peculiarity resides in the particular type of cell division observed, in two directions of the same plain, so that cells, during division, form tetrads. Interestingly, the closest relatives to pediococci are lactobacilli, the shape and mode of division of which are different (Simpson and Taguchi, 1995).

Moreover, the description of *Pediococcus siamensis* (▶ [Table 15.5](#)) has determined a rearrangement of the phylogenetic structure of the genus, with the recognition of two phylogenetically distinct subpopulations, one formed by

■ **Table 15.4**

List of valid names in the genus *Enterococcus* (updated at September 2008)

	Species of the genus <i>Enterococcus</i>
1	<i>Enterococcus aquimarinus</i>
2	<i>Enterococcus asini</i>
3	<i>Enterococcus avium</i>
4	<i>Enterococcus caccae</i>
5	<i>Enterococcus camelliae</i>
6	<i>Enterococcus canintestini</i>
7	<i>Enterococcus canis</i>
8	<i>Enterococcus casseliflavus</i>
9	<i>Enterococcus cecorum</i>
10	<i>Enterococcus columbae</i>
11	<i>Enterococcus devriesei</i>
12	<i>Enterococcus dispar</i>
13	<i>Enterococcus durans</i>
14	<i>Enterococcus faecalis</i>
15	<i>Enterococcus faecium</i>
16	<i>Enterococcus gallinarum</i>
17	<i>Enterococcus gilvus</i>
18	<i>Enterococcus haemoperoxidus</i>
19	<i>Enterococcus hermanniensis</i>
20	<i>Enterococcus hirae</i>
21	<i>Enterococcus italicus</i>
22	<i>Enterococcus malodoratus</i>
23	<i>Enterococcus moraviensis</i>
24	<i>Enterococcus mundtii</i>
25	<i>Enterococcus pallens</i>
26	<i>Enterococcus phoeniculicola</i>
27	<i>Enterococcus pseudoavium</i>
28	<i>Enterococcus raffinosus</i>
29	<i>Enterococcus ratti</i>
30	<i>Enterococcus saccharolyticus</i>
31	<i>Enterococcus silesiacus</i>
32	<i>Enterococcus sulfurous</i>
33	<i>Enterococcus termitis</i>
34	<i>Enterococcus thailandicus</i>
35	<i>Enterococcus villorum</i>

No species in the genus *Enterococcus* is under consideration for QPS status, principal species including probiotic strains are in bold and are described in the text

■ Table 15.5

List of valid names in the genus *Pediococcus* (updated at September 2008)

Species of the genus <i>Pediococcus</i>	
1	<i>Pediococcus acidilactici</i>
2	<i>Pediococcus cellicola</i>
3	<i>Pediococcus claussenii</i>
4	<i>Pediococcus damnosus</i>
5	<u><i>Pediococcus dextrinicus</i></u>
6	<i>Pediococcus ethanolidurans</i>
7	<i>Pediococcus inopinatus</i>
8	<i>Pediococcus parvulus</i>
9	<i>Pediococcus pentosaceus</i>
10	<i>Pediococcus siamensis</i>
11	<i>Pediococcus stilesii</i>

Species with QPS status are undelined, principal species including probiotic strains are in bold and are described in the text

Pediococcus claussenii, *Pediococcus pentosaceus* and *Pediococcus acidilactici*; the other including *Pediococcus damnosus*, *Pediococcus inopinatus*, *Pediococcus parvulus*, *Pediococcus ethanolidurans*, *Pediococcus stilesii*, *Pediococcus siamensis* and *Pediococcus cellicola*. *Pediococcus dextrinicus* is scarcely related with other species in the genus, and is more similar to lactobacilli (Felis and Dellaglio, 2007).

Pediococcus Acidilactici

The name of this first described species of the genus *Pediococcus* clearly indicates its ability to produce lactic acid. Strains of this species are mostly isolated from plant material (silage, cereal and potato mashes, barley, malt), but some strains have been isolated also from meat products. It produces both isomers of lactic acid from glucose and other carbohydrates, while it is usually unable to degrade maltose, a sugar usually associated with plant environment. Strains can also grow up to 50°C and in presence of sodium chloride at high concentration (10%). Genome GC content is in the range 38–44%.

The species has always shown high overall similarity with *Pediococcus pentosaceus* (see below), and this is confirmed also from their phylogenetic relatedness: they form a distinct clade in the phylogenetic tree of the genus, together with *Pediococcus claussenii*, and *Pediococcus stilesii*.

The type strain is DSM 20284^T (=LMG 11384^T), and no genome sequence data are available nor in progress (GOLD database).

Pediococcus Pentosaceus

As the name indicates, strains of this species are able to degrade pentoses (except strains of the formerly valid subsp. *intermedius*). It is very similar to *Pediococcus acidilactici* in terms of shape, culture conditions and physiological traits, and they are also isolated from almost the same plant niches. In principle the two species can be phenotypically distinguished on the basis of maltose degradation, and the slightly heat resistance (39–45°C but not 50°C) and genome GC content (35–39%), but reliable differentiation can only be obtained with molecular methods. Some strains are able to produce pediocin.

The type strain is ATCC 33316^T (=DSM 20336^T = LMG 11488^T), and genome data are available for a non-type strain (Gc00439).

15.3.2 Non LAB: Genera *Propionibacterium*, *Bacillus*, *Brevibacillus*, *Sporolactobacillus*, *Escherichia*

15.3.2.1 Genus *Propionibacterium*

The genus *Propionibacterium*, similarly to the genus *Bifidobacterium*, belongs to the Class *Actinobacteria* (Garrity et al., 2007c), which comprises high G + C content Gram-positive non sporeforming bacteria; its neighbor genera in the Family *Propionibacteriaceae* are *Brooklawnia*, *Jiangella*, *Luteococcus*, *Microlunatus*, *Propioniferax*, *Propionimicrobium*, and *Tessaracoccus*.

Propionibacteria take the name from the ability to produce propionic acid, acetic acid and carbon dioxide from carbohydrates and lactic acid; these characteristics are desirable for the production of some types of cheeses, e.g., the Swiss type, where propionibacteria are used as starter cultures. In general, morphology of propionibacteria might be very different: coccoid, bifid or even branched rods have been described, which vary also with the physiological state and culture conditions (Jan et al., 2007).

The genus comprises 12 species (🔗 [Table 15.6](#)) divided in two ecologically distinct groups, in terms of habitat/source of isolation: the “*acnes* group,” of human origin, and the “dairy” or “classical” propionibacteria, isolated from milk and dairy products. The latter group includes the only species proposed for QPS status, i.e., *Propionibacterium freudenreichii*. Other species have been isolated from different environments (e.g., *Propionibacterium cyclohexanicum*, from spoiled orange juice), and phylogenetically assigned to different subgroups,

■ Table 15.6

List of valid names in the genus *Propionibacterium* (updated at September 2008)

Species of the genus <i>Propionibacterium</i>	
1	<i>Propionibacterium acidipropionici</i>
2	<i>Propionibacterium acnes</i>
3	<i>Propionibacterium australiense</i>
4	<i>Propionibacterium avidum</i>
5	<i>Propionibacterium cyclohexanicum</i>
6	<u><i>Propionibacterium freudenreichii</i></u>
	<i>Propionibacterium freudenreichii</i> subsp. <i>freudenreichii</i>
	<i>Propionibacterium freudenreichii</i> subsp. <i>Shermanii</i>
7	<i>Propionibacterium granulosum</i>
8	<i>Propionibacterium innocuum</i>
9	<i>Propionibacterium jensenii</i>
10	<i>Propionibacterium microaerophilum</i>
11	<i>Propionibacterium propionicum</i>
12	<i>Propionibacterium thoenii</i>

Species with QPS status are underlined, principal species including probiotic strains are in bold and are described in the text

demonstrating that the correlation between phylogenetic and ecological structure of the genus is not obvious.

Propionibacterium Freudenreichii

Propionibacterium freudenreichii is the type species of the genus *Propionibacterium*, and it owes its name to the microbiologist von Freudenreich, who first described, with Orla-Jensen, the “*Bacterium acidi propionici* a.” It includes two subspecies, namely *Propionibacterium freudenreichii* subsp. *freudenreichii* and *Propionibacterium freudenreichii* subsp. *shermanii*. Strains of this species are commonly found in cheese, where are often used as starter cultures; to date only strains belonging to this species have probiotic properties confirmed in humans. As other propionibacteria, strains of this species can be rod-shaped or branched, can be observed as single cells or in pairs, or in groups, and grow anaerobically.

Propionibacterium freudenreichii belongs to the group of dairy species, but its closest phylogenetic relatives are two recently described species, i.e.,

Propionibacterium cyclohexanicum and *Propionibacterium australiense*, isolated from spoiled orange juice and granulomatous bovine lesions, respectively. The type strain of the subspecies is ATCC 6207^T, isolated from Swiss cheese.

Currently the genome sequencing for two strains in this species is ongoing, one for a non-type strain, not identified at the subspecies level (Gi00483), and a second one for the type strain of the subsp. *shermanii* CIP 103027^T (Gi 00772).

15.3.2.2 Spore-Forming Bacteria: Genera *Bacillus*, *Brevibacillus*, *Paenibacillus*, and *Sporolactobacillus*

The first description of genus *Bacillus* dates back to 1872 (Cohn) and it was based on two species, i.e., *Bacillus anthracis* and *Bacillus subtilis*. Nowadays, 147 species are ascribed to this genus (▶ [Table 15.7](#)), and many other species have also been transferred to other genera, i.e., *Alkalibacillus*, *Alicyclobacillus*, *Aneurinibacillus*, *Brevibacillus*, *Geobacillus*, *Gracilibacillus*, *Lysinibacillus*, *Marinibacillus*, *Paenibacillus*, *Pullulanibacillus*, *Salimicrobium*, *Sporolactobacillus*, *Sporosarcina*, *Ureibacillus*, *Virgibacillus*, and *Viridibacillus* (Garrity et al., 2007b).

According to a recent re-analysis of all archaea and bacteria described to date (Yarza et al., 2008) the genus *Bacillus* is sub-divided in at least eight phylogenetic groups, intermixed with other bacterial genera (see http://www.arb-silva.de/fileadmin/silva_databases/living_tree/LTP_tree_s93.pdf), in the taxonomic lineage of *Firmicutes*.

Members of the genus *Bacillus* (and related genera) are a heterogeneous group of aerobic rod shaped bacteria, producing lactic acid and other metabolites (e.g., carbon dioxide, diacetyl, bacteriocins), with a range of mole G + C genome content from 32 to 69%, with the most striking characteristic of producing endospores, a characteristic important also for probiotic application. Their heterogeneity results in difficult identification based on phenotypic but also on genetic tests (Sanders et al., 2003).

Bacillus species are commonly associated with soil, but frequent sources of isolation are also water, dust, and air, but are also easily found in the GIT of humans, mammals, aquatic animals and invertebrates, whether as contaminant or as residing *taxa*. Industrially, bacilli are employed for production of antibiotics, industrial chemicals, and enzymes. Also, the potential for food spoilage is known for some species. Furthermore, heat resistant spores are very problematic for some specific areas such as dried milk industry, and some species are known as possible agents in biological warfare in terroristic actions. However, *B. subtilis*

■ Table 15.7

List of valid names in the genus *Bacillus* (updated at September 2008) (Cont'd p. 622)

	Species of the genus <i>Bacillus</i>
1	<i>Bacillus acidicer</i>
2	<i>Bacillus acidicola</i>
3	<i>Bacillus aeolius</i>
4	<i>Bacillus aerius</i>
5	<i>Bacillus aerophilus</i>
6	<i>Bacillus agaradhaerens</i>
7	<i>Bacillus akibai</i>
8	<i>Bacillus alcalophilus</i>
9	<i>Bacillus algicola</i>
10	<i>Bacillus altitudinis</i>
11	<i>Bacillus alveayuensis</i>
12	<i>Bacillus amyloliquefaciens</i>
13	<i>Bacillus anthracis</i>
14	<i>Bacillus aquimaris</i>
15	<i>Bacillus arseniciselenatis</i>
16	<i>Bacillus arsenicus</i> Shivaji et al. (2005)
17	<i>Bacillus asahii</i>
18	<i>Bacillus atropheus</i>
19	<i>Bacillus aurantiacus</i>
20	<i>Bacillus azotoformans</i>
21	<i>Bacillus badius</i>
22	<i>Bacillus barbaricus</i>
23	<i>Bacillus bataviensis</i>
24	<i>Bacillus benzoovorans</i>
25	<i>Bacillus bogoriensis</i>
26	<i>Bacillus boroniphilus</i>
27	<i>Bacillus butanolivorans</i>
28	<i>Bacillus carboniphilus</i>
29	<i>Bacillus cellulolyticus</i>
30	<i>Bacillus cereus</i>
31	<i>Bacillus chagannorensis</i>
32	<i>Bacillus cibi</i>
33	<i>Bacillus circulans</i>
34	<i>Bacillus clarkii</i>
35	<i>Bacillus clausii</i>
36	<i>Bacillus coagulans</i>
37	<i>Bacillus coahuilensis</i>

■ **Table 15.7** (Cont'd p. 623)

	Species of the genus <i>Bacillus</i>
38	<i>Bacillus cohnii</i>
39	<i>Bacillus decisifrondis</i>
40	<i>Bacillus decolorationis</i>
41	<i>Bacillus drenthensis</i>
42	<i>Bacillus edaphicus</i>
43	<i>Bacillus endophyticus</i>
44	<i>Bacillus farraginis</i>
45	<i>Bacillus fastidiosus</i>
46	<i>Bacillus firmus</i>
47	<i>Bacillus flexus</i>
48	<i>Bacillus foraminis</i>
49	<i>Bacillus fordii</i>
50	<i>Bacillus fortis</i>
51	<i>Bacillus fumarioli</i>
52	<i>Bacillus funiculus</i>
53	<i>Bacillus galactosidilyticus</i>
54	<i>Bacillus gelatini</i>
55	<i>Bacillus gibsonii</i>
56	<i>Bacillus ginsengihumi</i>
57	<i>Bacillus halmopalus</i>
58	<i>Bacillus halodurans</i>
59	<i>Bacillus hemicellulosilyticus</i>
60	<i>Bacillus herbersteinensis</i>
61	<i>Bacillus horikoshii</i>
62	<i>Bacillus horti</i>
63	<i>Bacillus humi</i>
64	<i>Bacillus hwajinpoensis</i>
65	<i>Bacillus idriensis</i>
66	<i>Bacillus indicus</i>
67	<i>Bacillus infantis</i>
68	<i>Bacillus infernus</i>
69	<i>Bacillus insolitus</i>
70	<i>Bacillus isabeliae</i>
71	<i>Bacillus jeotgali</i>
72	<i>Bacillus koreensis</i>
73	<i>Bacillus kribbensis</i>
74	<i>Bacillus krulwichiae</i>
75	<i>Bacillus lehensis</i>

■ **Table 15.7** (Cont'd p. 624)

	Species of the genus <i>Bacillus</i>
76	<i>Bacillus lentus</i>
77	<i>Bacillus licheniformis</i>
78	<i>Bacillus litoralis</i>
79	<i>Bacillus luciferensis</i>
80	<i>Bacillus macauensis</i>
81	<i>Bacillus macyae</i>
82	<i>Bacillus mannanilyticus</i>
83	<i>Bacillus marisflavi</i>
84	<i>Bacillus massiliensis</i>
85	<i>Bacillus megaterium</i>
86	<i>Bacillus methanolicus</i>
87	<i>Bacillus mojaviensis</i>
88	<i>Bacillus mucilaginosus</i>
89	<i>Bacillus muralis</i>
90	<i>Bacillus murimartini</i>
91	<i>Bacillus mycoides</i>
92	<i>Bacillus nealsonii</i>
93	<i>Bacillus niabensis</i>
94	<i>Bacillus niacini</i>
95	<i>Bacillus novalis</i>
96	<i>Bacillus odysseyi</i>
97	<i>Bacillus okhensis</i>
98	<i>Bacillus okuhidensis</i>
99	<i>Bacillus oleronius</i>
100	<i>Bacillus oshimensis</i>
101	<i>Bacillus panaciterrae</i>
102	<i>Bacillus patagoniensis</i>
103	<i>Bacillus plakortidis</i>
104	<i>Bacillus pocheonensis</i>
105	<i>Bacillus polygoni</i>
106	<i>Bacillus pseudocaliphilus</i>
107	<i>Bacillus pseudofirmus</i>
108	<i>Bacillus pseudomycooides</i>
109	<i>Bacillus psychrodurans</i>
110	<i>Bacillus psychrosaccharolyticus</i>
111	<i>Bacillus psychrotolerans</i>
112	<i>Bacillus pumilus</i>
113	<i>Bacillus pycnus</i>

■ Table 15.7

	Species of the genus <i>Bacillus</i>
114	<i>Bacillus qingdaonensis</i>
115	<i>Bacillus ruris</i>
116	<i>Bacillus safensis</i>
117	<i>Bacillus salarius</i>
118	<i>Bacillus saliphilus</i>
119	<i>Bacillus schlegelii</i>
120	<i>Bacillus selenitarsenatis</i>
121	<i>Bacillus selenitireducens</i>
122	<i>Bacillus seohaeanensis</i>
123	<i>Bacillus shackletonii</i>
124	<i>Bacillus silvestris</i>
125	<i>Bacillus simplex</i>
126	<i>Bacillus siralis</i>
127	<i>Bacillus smithii</i>
128	<i>Bacillus soli</i>
129	<i>Bacillus sonorensis</i>
130	<i>Bacillus sporothermodurans</i>
131	<i>Bacillus stratosphericus</i>
132	<u><i>Bacillus subterraneus</i></u>
133	<i>Bacillus subtilis</i>
	<i>Bacillus subtilis</i> subsp. <i>spizizenii</i>
	<i>Bacillus subtilis</i> subsp. <i>subtilis</i>
134	<i>Bacillus taeanensis</i>
135	<i>Bacillus tequilensis</i>
136	<i>Bacillus thermantarcticus</i>
137	<i>Bacillus thermoamylovorans</i>
138	<i>Bacillus thermocloacae</i>
139	<i>Bacillus thioparans</i>
140	<i>Bacillus thuringiensis</i>
141	<u><i>Bacillus tusciae</i></u>
142	<i>Bacillus vallismortis</i>
143	<i>Bacillus vedderi</i>
144	<i>Bacillus vietnamensis</i>
145	<i>Bacillus vireti</i>
146	<i>Bacillus wakoensis</i>
147	<i>Bacillus weihenstephanensis</i>

Species with QPS status are underlined, principal species including probiotic strains are in bold and are described in the text

is traditionally used in Eastern countries to produce specific foods, which demonstrates its safe use for food application, together with other species. In fact, members of the genus *Bacillus* are used as probiotics, where one of the main advantages over classical lactobacilli is the availability of endospores, which may be stored desiccated almost indefinitely (Fritze and Claus, 1995).

Spore-forming bacteria proposed for probiotics production belong mainly to the species *Bacillus cereus*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus indicus*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus subtilis*, “*Bacillus laterosporus*,” “*Bacillus laevolacticus*,” “*Bacillus polymyxa*” and “*Bacillus polyfermenticus*.” Species names reported in inverted commas are not valid either because they are not updated (“*B. laterosporus*” is now *Brevibacillus laterosporus*, “*Bacillus polymyxa*” corresponds to *Paenibacillus polymyxa*, and “*Bacillus laevolacticus*” belongs to the genus *Sporolactobacillus*, with the same species name) or they have not been formally described (“*Bacillus polyfermenticus*”). Some details on the genera *Brevibacillus* and *Sporolactobacillus* are given below, before species explanations.

The genus *Brevibacillus*, the name meaning short rod, has been described in 1996 to accommodate, on the basis of phylogenetic relatedness, ten species of Gram-positive, motile, aerobic spore-forming bacteria previously belonging to the genus *Bacillus* (Shida et al., 1996). After publication of that new genus name, other four species have been described, i.e., *Brevibacillus ginsengsoli*, *Brevibacillus invocatus*, *Brevibacillus levickii*, and *Brevibacillus limnophilus* (▶ [Table 15.8](#)). Ecologically, *Brevibacillus* inhabit the same environments as *Bacillus*. *Brevibacillus brevis* is the type species of the genus.

Species in the genus *Paenibacillus* are facultatively anaerobic or strictly aerobic bacteria, rod shaped, generally Gram-positive but also variable, usually motile, almost all catalase positive. They have different degrading abilities, excreting diverse proteolytic and/or extracellular polysaccharide-hydrolyzing enzymes. The G + C contents range from 45 to 54 mol%. The type species is *Paenibacillus polymyxa*. The genus groups more than 90 species, none of them has been proposed for QPS status, strains considered probiotics seem to belong to *Paenibacillus polymyxa* only.

The genus *Sporolactobacillus* comprises six species of catalase-negative, facultative anaerobic or microaerophilic endosporeformers (▶ [Table 15.9](#)). Due to the metabolic similarities with genuine lactic acid bacteria, species of this genus were originally thought to belong to the genus *Lactobacillus*, but then assigned to *Bacillus*, due to their ability to form spores, and eventually described as novel genus, in the Family *Sporolactobacillaceae* (Garrity et al., 2007b). The type species for *Sporolactobacillus* is *Sporolactobacillus inulinus*. Members of the genus have been isolated from chicken feed, soil, dairy products, and pickle.

■ **Table 15.8**

List of valid names in the genus *Brevibacillus* (updated at September 2008)

	Species of the genus <i>Brevibacillus</i>
1	<i>Brevibacillus agri</i>
2	<i>Brevibacillus borstelensis</i>
3	<i>Brevibacillus brevis</i>
4	<i>Brevibacillus centrosporus</i>
5	<i>Brevibacillus choshinensis</i>
6	<i>Brevibacillus formosus</i>
7	<i>Brevibacillus ginsengisoli</i>
8	<i>Brevibacillus invocatus</i>
9	<i>Brevibacillus laterosporus</i>
10	<i>Brevibacillus levickii</i>
11	<i>Brevibacillus limnophilus</i>
12	<i>Brevibacillus parabrevis</i>
13	<i>Brevibacillus reuszeri</i>
14	<i>Brevibacillus thermoruber</i>

No species has been proposed for QPS status. Species including proposed probiotic strains are in bold and are described in the text

■ **Table 15.9**

List of valid names in the genus *Sporolactobacillus* (updated at September 2008)

	Species of the genus <i>Sporolactobacillus</i>
1	<i>Sporolactobacillus inulinus</i>
2	<i>Sporolactobacillus kofuensis</i>
3	<i>Sporolactobacillus lactosus</i>
4	<i>Sporolactobacillus laevolacticus</i>
5	<i>Sporolactobacillus nakayamae</i>
	<i>Sporolactobacillus nakayamae</i> subsp. <i>nakayamae</i>
	<i>Sporolactobacillus nakayamae</i> subsp. <i>Racemicus</i>
6	<i>Sporolactobacillus terrae</i>

No species has been proposed for QPS status. Species including proposed probiotic strains are in bold and are described in the text

Bacillus Cereus

The species *Bacillus cereus*, the species name meaning wax-coloured, is associated with food poisoning, due to the production of toxins, however strains belonging to the “var. *vietnami*” have been indicated as probiotics (Duc et al., 2004).

The indication of a variety (the “var.”) has no meaning in bacterial taxonomy. In fact this name has been assigned to one strain exhibiting physiological traits similar to *Bacillus cereus* but apparently not phylogenetically related to it. According to Hoa et al. (2000), these data could justify the description of a novel species. For the time being, this description has not appeared, therefore the name cannot be considered valid. Also, another organism attributed with probiotic properties is “*Bacillus toyoi*” or “*Bacillus cereus* var. *toyoi*,” used mostly for animals (e.g., De Cupere et al., 1992; Scharek et al., 2007), but similar considerations presented for “var. *vietnami*” apply.

In general, *Bacillus cereus* is motile, hemolytic on blood agar, penicillin resistant. Its closest relatives are *Bacillus mycoides*, *Bacillus weihenstephanensis*, *Bacillus thuringensis*, *Bacillus anthracis* and *Bacillus pseudomycooides*. According to the tree obtained by Yarza et al. (2008) this group of bacteria seems to be more closely related with the genus *Gemella* than with other *Bacilli*.

Type strain of *Bacillus cereus* is DSM 31^T (=ATCC 14579^T) which has also been sequenced (Gc00135) together with other strains, already completed (Gc00617, Gc00215, and Gc00173) or ongoing (40 more), due to the clinical interest for the species.

Bacillus Clausii

Bacillus clausii, described in 1995 with other alkaliphilic species in the genus *Bacillus*, is named after Claus, a German microbiologist who made fundamental contribution to the study of bacilli, and it groups bacteria isolated from soil. The closest phylogenetic relatives are *Bacillus oshimensis*, *Bacillus lehensis*, *Bacillus patagoniensis*, *Bacillus gibsonii*, *Bacillus murimartini*, and *Bacillus plakortidis*. As a species, it is characterized by the production of catalase and oxidase, is able to reduce nitrate and to hydrolyze starch and gelatin, finally, it grows between 30 and 50°C and with up to 10% sodium chloride. Genome GC content is around 43 mol% (Nielsen et al., 1995). The type strain, isolated from Garden soil, is ATCC 700160^T (=DSM 8716^T). Genome sequencing projects exist for strains in the species, one complete (Gc00228), and one incomplete (Gi00061), strains chosen for sequencing are not the type strain.

Bacillus Coagulans

Bacillus coagulans was first described in 1915 isolated from spoiled canned milk, in which it had caused coagulation (from which the names) is a thermotolerant and microaerophilic species, associated to spoilage of foods such as milk

products, vegetables and fruits. Also *Bacillus coagulans* is exploited industrially, e.g., as a source of thermostable enzymes, and it is employed also as growth promoting additive in food and feed, often under the invalid name of “*Lactobacillus sporogenes*.” Different morphologies of the cells, spore surfaces and sporangia have been reported, complicating the recognition of *Bacillus coagulans* as a single species. An emended description of the species based on a polyphasic approach and on 31 strains confirmed the heterogeneity of the species (De Clerck et al., 2004), the strains of which have been isolated from different environments, suggesting high flexibility. The closest phylogenetic relatives are *Bacillus oleronius*, *Bacillus sporothermodurans*, *Bacillus acidicola*, and *Bacillus ginsenghumi*, which, as a clade, appear to be quite unrelated to *Bacillus subtilis* group. The type strain is ATCC 7050^T (=LMG 6326^T = DSM 1^T) isolated from evaporated milk, but other strains have been isolated from soil. A genome project is ongoing (Gi01001) for a non-type strain.

Bacillus Licheniformis

The first description of the *taxon* called today *B. licheniformis* dates back to 1898, and it owes its name to its shape, the name meaning lichen-shaped. As other bacilli, it is Gram-positive, microaerophilic, and motile. *Bacillus licheniformis* is an industrially important strain as it produces enzymes and bacteriocins. Strains of *Bacillus licheniformis* have been isolated from soil, milk, water, but also septic wounds, and are generally considered safe. However, Salkinoja-Salonen et al. (1999) and other studies have reported on the toxigenic activity of *Bacillus licheniformis*, *Bacillus pumilus*, and *Bacillus subtilis*. Interestingly, these three species, proposed for probiotic application, also belong to the same phylogenetic group.

The type strain is ATCC 14580^T (=DSM 13^T = LMG 12363^T), and genome data for DSM 13^T have been obtained by two groups independently (Gc00213, and Gc00221).

Bacillus Pumilus

Strains of this species, the name of which means “small,” are used for enzyme production on industrial scale, and for many other applications.

Strains in this species are aerobic and motile, positive for catalase as well as for oxidase, beta-galactosidase and amylase. Acid production is observed from glucose, arabinose, mannitol and xylose. As for pH it is a neutrophilic species, while, considering heat resistance of vegetative cells, it is mesophilic. This species is also associated to food poisoning due to toxin production. It belongs to *Bacillus*

subtilis phylogenetic group (see below). Its type strain is ATCC 7061^T (=DSM 27^T = LMG 18928^T). Genome sequences for four strains are completed or in progress, including one for the type strain (Gc00656, Gi03241, Gi01901, and Gi00674).

Bacillus Subtilis

The slender shape of these rods, less than 1 µm wide, gives the names to the *taxon*, which is the type species of the genus. As anticipated, *Bacillus subtilis* is the classical model organism for genetic research in Gram-positive bacteria, but it is also widely used in traditional and industrial fermentation processes as well as in agriculture. Phylogenetically it is very closely related to species *Bacillus altitudinis*, *Bacillus aerius*, *Bacillus aerophilus*, *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus licheniformis*, *Bacillus mojavensis*, *Bacillus pumilus*, *Bacillus safensis*, *Bacillus sonorensis*, *Bacillus stratosphericus*, *Bacillus vallismortis*, and *Bacillus velezensis* but also to *Brevibacterium halotolerans*. Its sporangia are not swollen, and spores are ellipsoidal. It grows between 15–20 and 45–55°C, with an optimum at 28–30°C. It also grows in presence of 7% sodium chloride. It is a strictly aerobic and catalase positive species, able to hydrolyse starch and casein.

Recently, a second subspecies has been described, i.e., subsp. *spizizenii*, named after American bacteriologist Spizizen, on the basis of significant sexual isolation found between two genotypically distinguishable populations within the species and DNA-DNA hybridization levels in the range of 58–68%. These values are thus just below the threshold usually applied for the delineation of species (70%) but, due to the high overall similarity of this second group of bacteria with the type strain of *Bacillus subtilis* the two populations were retained in the same species (Nakamura et al., 1999). Type strains are ATCC 6051^T (=DSM 10^T = LMG 7135^T), for subsp. *subtilis*, and NRRL B-23049^T (=LMG 19156^T), for subsp. *spizizenii*. Genome sequence data are available or in progress for 10 strains (Gc00010, Gi01245, Gi03233, Gi01244, Gi01246, Gi03234, Gi03274, Gi03229, Gi03230, and Gi03239).

Brevibacillus Laterosporus (Formerly “Bacillus laterosporus”)

Brevibacillus laterosporus strains take their name from the lateral position of spores. These bacteria show low level of larvicidal activity, and have also been associated with human infections. As for temperature for growth, it ranges between 15 and 50°C, with an optimum at 30°C. The species reduces nitrate and is capable of casein and gelatin hydrolysis, but not of starch.

Phylogenetically the genus appears quite homogeneous. Type strain is ATCC 4517^T (=DSM 25^T) while no sequence data are in progress at the moment, according to lists in GOLD database.

Paenibacillus Polymyxa (Formerly “Bacillus polymyxa”)

As anticipated, *Paenibacillus polymyxa* is the type strain of the genus. Its closest phylogenetic relatives are *Paenibacillus kribbensis*, *Paenibacillus peoriae*, *Paenibacillus jamilae*, *Paenibacillus brasiliensis*, and *Paenibacillus terrae*. It is able to hydrolyze pectin and xylan and fix nitrogen, and it produces acid from various sugars. Another interesting characteristic is the production of slime, which gives the origin to the name, which literally means “much slime.” Its habitat is probably soil. The type strain is ATCC 842^T, and genome sequence data are available (Gi00423) for a non-type strain.

Sporolactobacillus Inulinus

Sporolactobacilli, in general, and species *inulinus* in particular, display intermediate characteristics with respect to *Bacillus* and *Lactobacillus*. In common with the former is the ability to form spores, and the presence of diaminopimelic acid as cell wall component. Similarly to the latter, they are catalase negative, micro-aerophilic and produce lactic acid from glucose through homolactic fermentation. The lactic acid isomer produced is D (-). Genome GC content is 38–39%. The type strain is ATCC 15538^T (=DSM 20348^T = LMG 11481^T), and no genome sequence data appear to be in progress in GOLD database.

Sporolactobacillus Laevolacticus

The taxonomic status of this motile bacterium producing D(-) – lactic acid has been questioned for a long time, but only recently (Andersch et al., 1994; Hatayama et al., 2006) its description has been validly published, including motile bacteria with diaminopimelic acid in the cell wall. The species is facultatively anaerobic, catalase positive, and mesophilic, with 40°C as the maximum temperature for growth. It hydrolyzes starch and degrades sugars through homolactic fermentation. Strains have been isolated from rhizospheres of plants. The type strain is ATCC 23492^T (=DSM 442^T = LMG 6329^T), and no sequence data are available.

Notably, *Bacillus polyfermenticus* is not a validly published name. Therefore, even if research reports on its probiotic properties have been published, no reliable characteristics can be assigned to this name, and no strain can be indicated as reference point.

15.3.2.3 Genus *Escherichia*

The genus *Escherichia* belongs to the Phylum *Proteobacteria*, Class *Gammaproteobacteria*, Order *Enterobacteriales*, Family *Enterobacteriaceae*. Genera in the same family are: *Arsenophonus*, *Brenneria*, *Buchnera*, *Budvicia*, *Buttiauxella*, *Cedecea*, *Citrobacter*, *Dickeya*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Ewingella*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Leminorella*, *Moellerella*, *Morganella*, *Obesumbacterium*, *Pantoea*, *Pectobacterium*, *Phlomobacter*, *Photorhabdus*, *Plesiomonas*, *Pragia*, *Proteus*, *Providencia*, *Rahnella*, *Raoultella*, *Saccharobacter*, *Salmonella*, *Samsonia*, *Serratia*, *Shigella*, *Sodalis*, *Thorsellia*, *Tatumella*, *Trabulsiella*, *Wigglesworthia*, *Xenorhabdus*, *Yersinia*, and *Yokenella* (Garrity et al., 2007d).

Escherichia includes six species of Gram-negative rods, chemo-organotrophic with both oxidative and fermentative metabolism, catalase-positive which produce acid and gas from glucose.

From a taxonomic standpoint, the genus should also include the four species of the genus *Shigella*, which are retained separate only for historical and clinical reasons (Lan and Reeves, 2002).

Escherichia coli is the best known species of the genus, and it is a widespread commensal of the lower intestinal tract of humans and other vertebrates. Clones of *Escherichia coli* cause intestinal and extra-intestinal diseases with devastating effects on the host. However, as a commensal, some *Escherichia coli* strains are also used as probiotics. The type strain of *Escherichia coli* is ATCC 11775^T (=LMG 2092^T = DSM 30083^T = JCM 1649^T). It was isolated from urine but it has not been sequenced although about 71 genome sequences are reported as completed (13) or ongoing (58) projects for the species *Escherichia coli*, not considering *Shigella*.

In a recent genomic study, Willenbrock et al. (2007) analyzed the genomic content of four probiotic strains by microarray. Results showed that the probiotic strains were most similar, in terms of gene pool, with *Escherichia coli* K-12 strains, and with H10407, which is an enterotoxigenic strain, the virulence of which is plasmid-encoded. Some virulence related genes were also detected in these isolates, indicating that both pathogenic and non-pathogenic *Escherichia coli* strains use common strategies for adaptation to their niche. Finally, genetic flexibility was witnessed by the presence of strain-specific phage genes, transposases, insertion elements and mobile-elements-related genes. These data cannot be used in devising a taxonomic scheme as they are not stable elements, but are of utmost importance for characterization at the strain level.

15.3.3 Yeasts

Yeasts are unicellular eukaryotic microorganisms. The strains used as probiotics are referred to as “*Saccharomyces boulardii*” however this species name has no meaning in taxonomy as it is a synonym for *Saccharomyces cerevisiae* (Edwards-Ingram et al., 2004; Hennequin et al., 2001; McCullough et al., 1998; Mitterdorfer et al., 2002).

According to Kurtzman and Fell (1999), the genus *Saccharomyces* includes 14 species. However more recently, Kurtzman (2003), on the basis of Multigene Sequence Analysis, proposed a new *Saccharomyces* genus that includes only seven of the previous species (*Saccharomyces cariocanus*, *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomyces mikatae*, *Saccharomyces kudriavzevii*, *Saccharomyces paradoxus*, and *Saccharomyces pastorianus*); the rest of the previous species are in a new genus, namely *Kazachstania*. *Saccharomyces cerevisiae* is the type species of the genus.

Considering the taxonomic lineage, the genus *Saccharomyces* is grouped in the Domain Eukaryota, Kingdom Fungi, Phylum Ascomycota, Subdivision Saccharomycotina, Class Saccharomycetes, Order Saccharomycetales, Family Saccharomycetaceae. It can be noted that names above the genus level are not written in italics, according to the rules of the International Code of Botanical Nomenclature (McNeill et al., 2006) and differently from bacterial nomenclature.

Cells of *Saccharomyces cerevisiae* reproduce vegetatively by multilateral budding (a characteristic of the Class Saccharomycetes), and are transformed directly to asci, containing ascospores, when grown on acetate agar. Also, a characteristic of the genus *Saccharomyces* is the vigorous fermentation of sugars, which is the desired characteristic of strains of *Saccharomyces cerevisiae* (and other species) used for food production, from breadmaking to production of alcoholic beverages from a wide range of vegetable raw materials. Besides its commercial importance, *Saccharomyces cerevisiae* is a very important model system for molecular biology and genetics, as the basic cellular mechanisms of this simple eukaryote are largely conserved also in higher organisms, including mammals. The name of the anamorph for *Saccharomyces cerevisiae* is *Candida robusta*, and a large number of synonyms have been determined. The type strain is CBS 1171^T (=ATCC 18824^T = DBVPG 6173^T = NRRL Y-12632^T), isolated in a Dutch brewery (Vaughan-Martini and Martini, 1999). Genome

sequence for one strain has been determined (Gc00006) but 35 others are ongoing.

15.4 Summary

- Names are the results of taxonomic procedures, i.e., extensive and time consuming characterization of organisms under different aspects (genetic, physiology, etc) in order to define their diversity.
- Taxonomic studies of microorganisms have always been dependent on scientific and technological advancements and development of novel techniques of investigation, due to their extremely small size. Application of new techniques can highlight novel traits and therefore can modify the understanding of diversity of organisms, thus changing also names.
- Correct names of organisms are essential as they (i) allow retrieving all the updated information on *taxa*, (ii) constitute a standard for the unambiguous identification of bacteria, both for scientific purposes, for definition of lists of safe and pathogenic bacteria, and for reliable commercial information on microorganisms.
- Taxonomic traits, i.e., characteristics useful for characterization of genera, species and subspecies can be different in different organisms, as they depend also on ecology and evolution. Therefore, classification of different groups of bacteria could be slightly different and it has to be considered an “agreement among experts.” On the other hand, nomenclature, i.e., the assignment of names to recognized taxa, is strictly regulated, to ensure clarity.
- Probiotic properties are strains specific and not species specific, but an accurate identification at species level is essential to have more information on the strains.

List of Abbreviations

<i>EFSA</i>	European Food Safety Authority
<i>GIT</i>	gastro-intestinal tract
<i>LAB</i>	lactic acid bacteria
<i>QPS</i>	qualified presumption of safety

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