# BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY

## On Using the Manual

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THE MANUAL is meant to assist in the identification of bacteria. No attempt has been made to provide a complete hierarchy, as in previous editions, because a complete and meaningful hierarchy is impossible. Instead THE MANUAL is presented in 19 parts based on a few readily determined criteria.

Each part bears a vernacular name and sometimes that of a taxon. All accepted genera have been placed in what seems the most appropriate part, although allocation of some genera presents difficulties; for example, Part 1 ("Phototrophic Bacteria") includes three genera of budding bacteria which are mostly in Part 4; the chemolithotrophic genus *Nitrobacter* (Part 12) also produces buds.

In Parts 1, 5, 11, 13, 14 and 18 all accepted genera have been placed in families (there may be an occasional exception covered in an addendum). In Parts 3 and 4 no attempt has been made to indicate families. In other parts the families are followed by "Genera of Uncertain Affiliation." While these genera belong in the part, as defined, they have not been accepted into any of the families and cannot themselves be grouped into families on the information now available.

# PART 16

# GRAM-POSITIVE, ASPOROGENOUS, ROD-SHAPED BACTERIA*

## FAMILY I. **LACTOBACILLACEAE** WINSLOW, BROADHURST, BUCHANAN, KRUMWIEDE, ROGERS AND SMITH 1917, 561

Lac.to.ba.cil.lac'e.ae. M.L. masc.n. *Lactobacillus* type genus of the family; *-aceae* ending to denote a family; M.L. fem.pl.n. *Lactobacillaceae* the *Lactobacillus* family.

Straight or curved rods usually occurring singly or in chains. Non-motile, rare strains motile. Gram-positive. Anaerobic or facultative. Complex organic nutritional requirements. Highly saccharoclastic. At least half the end product carbon from carbohydrate metabolism is lactate. Lactate is not attacked anaerobically. Catalase negative (no porphyrin in apoprotein). Benzidine reaction negative. Pathogenicity unusual. Found in fermenting animal and plant products where carbohydrates are available; also found in the mouth, vagina and intestinal tracts of various warm-blooded animals, including man.

### *Genus I.* **Lactobacillus** *Beijerinck 1901, 212. Nom. cons.* Opin. 38, Jud. Comm. 1971, 104

MORRISON ROGOSA

Lac.to.ba.cil'lus. L. n. *lac, lactis* milk; L. dim.n. *bacillus* a small rod; M.L. masc.n. *Lactobacillus* milk rodlet.

Rods, varying from long and slender to short coccobacilli. Chain formation common, particularly in later logarithmic phase of growth. Motility unusual; when present, by peritrichous flagella. Non-sporing. Gram-positive becoming Gram-negative with increasing age and acidity. Some strains exhibit bipolar bodies, internal granulations or a barred appearance with the Gram reaction or methylene blue stain.

Metabolism fermentative even though growth generally occurs in air; some are strict anaerobes on isolation.

Characteristically saccharoclastic; glucose fermented decreasing pH 1 or more units. At least half of end product carbon is lactate. Lactate is not fermented. Additional products may be acetate, formate, succinate, $CO_2$ or ethanol. Volatile acids with more than 2 carbon atoms are not produced.

Nitrate reduction highly unusual and then only where terminal pH is poised above 6.0. Gelatin not liquefied. Casein not digested but minute amounts of soluble nitrogen produced by some strains of some species. Indole and $H_2S$ not produced.

Catalase and cytochrome negative (porphyrins absent); however, rare strains decompose peroxide by a pseudocatalase (porphyrin not present); benzidine reaction negative.

Pigment production rare; if present, yellow or orange to rust or brick red.

Complex nutritional requirements for amino acids, peptides, nucleic acid derivatives, vitamins, salts, fatty acids or fatty acid esters and fermentable carbohydrates. Nutritional requirements are generally characteristic for each species.

Surface growth on solid media often enhanced by anaerobiosis and 5–10% $CO_2$.

Temperature range 5–53 C; optimum generally 30–40 C.

Aciduric, optimal pH usually 5.5–5.8 or less, and

***Editorial Note.** The division of genera between Part 16 and the first section of Part 17 is arbitrary and readers should consult both. Some genera have traditionally been associated with the actinomycetes and are so treated in this edition.

generally growing at 5.0 or less; at neutral or initial alkaline reactions lag phase may be lengthened or total growth yield reduced.

Found in dairy products and effluents, grain and meat products, water, sewage, beer, wine, fruits and fruit juices, pickled vegetables, sourdough and mash; also parasitic in the mouth, intestinal tract and vagina of many homothermic animals including man. Pathogenicity is highly unusual.

The G + C content of the DNA ranges from 34.7 ± 1.4–53.4 ± 0.5 moles % (buoyant density).

Type species: *Lactobacillus delbrueckii* (Leichmann) Beijerinck 1901, 229. Opin. 38, Jud. Comm. 1971, 104.

*Key to the species of genus* **Lactobacillus**

I. Homofermentative. Lactic acid is the major product from glucose (generally 85% or more).

A. No gas from glucose or gluconate; ribose not fermented, thiamine not required; aldolase activity; D- or L- or DL-lactic acid produced; G + C = 34.7–50.8%; generally grows at 45 C or higher, generally not at 20 C and not at 15 C. Colonies normally rough becoming smooth and compact in the presence of Tween 80 or sodium oleate (Rogosa and Mitchell, 1950).

1. Produce D(−)-lactic acid.*

1. *L. delbrueckii*
2. *L. leichmannii*
3. *L. jensenii*

4. *L. lactis*
5. *L. bulgaricus*

2. Produce DL-lactic acid.

6. *L. helveticus*
7. *L. acidophilus*

3. Produce mainly L(+)- and very small amounts of D(−)-lactic acid.*

8. *L. salivarius*

TABLE 16.1—*Carbohydrate reactions of the species of genus* **Lactobacillus**

| | 1. *L. delbrueckii* | 2. *L. leichmannii* | 3. *L. jensenii* | 4. *L. lactis* | 5. *L. bulgaricus* | 6. *L. helveticus* | 7. *L. acidophilus* | 8. *L. salivarius* |
|---|---|---|---|---|---|---|---|---|
| Amygdalin | −[a] | + | + | − | − | − | + | − |
| Arabinose | − | − | − | − | − | − | − | − |
| Cellobiose | − | + | + | − | − | − | + | − |
| Fructose | + | + | + | + | + | ⁄ | + | + |
| Galactose | ⁄ | −w | + | + | + | + | + | + |
| Glucose (acid) | + | + | + | + | + | + | + | + |
| Glucose (gas) | − | − | − | − | − | − | − | − |
| Gluconate | − | − | − | − | − | − | − | − |
| Lactose | − | + | − | + | + | + | + | + |
| Maltose | d | + | + | + | − | + | + | + |
| Mannitol | − | − | − | − | − | − | − | + |
| Mannose | + | + | + | + | − | ⁄ | + | + |
| Melezitose | − | − | − | − | − | − | − | − |
| Melibiose | − | − | − | − | − | − | d | + |
| Raffinose | − | − | − | − | − | − | d | + |
| Rhamnose | − | − | − | − | − | − | − | * |
| Ribose | − | − | − | − | − | − | − | − |
| Salicin | − | + | + | + | − | − | + | * |
| Sorbitol | − | − | − | − | − | − | − | + |
| Sucrose | + | + | + | + | − | − | + | + |
| Trehalose | − | + | + | + | − | * | + | + |
| Xylose | − | − | − | − | − | − | − | − |
| Esculin | − | + | + | ⁄ | − | − | + | * |

[a] The symbols used are: + = positive reaction by 90% or more strains; d = some strains +, others − (about 89–11% positive); − = negative reaction by most strains (90% or more); ( ) = delayed reaction; w = weak reaction; ⁄ = weak, slow or negative; * = see text; 0 = not tested. Combinations of symbols, e.g. (−) = rarely positive, and then slowly; −w = negative or weak reaction.

5. **Lactobacillus bulgaricus** (Orla-Jensen) Rogosa and Hansen 1971, 181. (*Thermobacterium bulgaricum* Orla-Jensen 1919, 164.)

Names of organisms whose relationship to *L. bulgaricus* is uncertain: *Lactobacillus longus* Beijerinck 1901, 217; Bacille A Grigoroff 1905, 716; *Bacillus bulgaricus* Luerssen and Kühn 1907, 241; *Bacterium bulgaricum* (Luerssen and Kühn) Buchanan and Hammer 1915, 250; *Acidobacterium bulgaricum* (Luerssen and Kühn) Schlirf 1925, 116; *Plocamobacterium bulgaricum* (Luerssen and Kühn) Lehmann and Neumann 1927, 511; *Bacterium giogurt* de'Rossi 1927, 743; *Lactobacterium bulgaricum* (Luerssen and Kühn) Krasil'nikov 1949, 212.

bul.ga'ri.cus. M.L. adj. *bulgaricus* Bulgarian.

This species is closely related to *Lactobacillus lactis*, being morphologically indistinguishable, producing the same amount of D(−)-lactic acid in milk, having the same general cell wall structure and group antigen, including glycerol teichoic acid and a peptidoglycan of the L-lysine-D-aspartate type, apparently identical lactic acid dehydrogenases and similar G + C moles % in the DNA (50.3). The only significant difference is that *L. bulgaricus* ferments fewer sugars than *L. lactis* (Table 16.1). The latter might be a mutant or variant of the former.

Type strain: ATCC 11842 (original strain No. 14 from Orla-Jensen (1919) (Rogosa and Hansen, 1971)).

Comments: Previous descriptions in THE MANUAL were composites of two organisms, one producing D(−)- and the other producing DL-lactic acid; sugar fermentations of this "organism" reported from various sources were in conflict; from study of the actual strains on which THE MANUAL's descriptions were based, it is definite that the two organisms involved are *L. bulgaricus* and *L. jugurti* (the latter a maltose negative variant of *L. helveticus*). In DNA-DNA hybridization experiments there is no reassociation of *L. bulgaricus* DNA and *L. jugurti* DNA (Simonds *et al.*, 1971). These, and sometimes *L. lactis* and other lactobacilli, often occur simultaneously in such sour milks as yogurt, etc. (see Rogosa and Hansen (1971) for documentation).

7. **Lactobacillus acidophilus** (Moro) Hansen and Mocquot 1970, 326. (*Bacillus acidophilus* Moro 1900, 115; *Thermobacterium intestinale* Orla-Jensen, Orla-Jensen and Winther 1936, 331.)

a.ci.do'phi.lus. L. adj. *acidus* sour; M.L. neut.n. *acidum* acid; Gr. adj. *philus* loving; M.L. adj. *acidophilus* acid-loving.

Rods with rounded ends, generally 0.6–0.9 by 1.5–6 μm, occurring singly, in pairs and in short chains. Non-motile. Non-flagellated.

Colony usually rough. Microscopic examination generally reveals twisted or fuzzy filamentous projections with dark felt-like mass in the center. Deep colonies are irregularly shaped with radiate or ramified projections. No characteristic pigment.

Glycogen fermented by some strains and generally weakly. Some strains ferment melibiose, raffinose or both. Homofermentative, producing DL-lactic acid. Generally less than 10% of other carbohydrate fermentation products.

Ammonia not produced from arginine. Acidity and coagulation of milk variable; acidity varying from 0.3–1.9% lactic acid.

Cell wall peptidoglycan is of the L-lysine-D-aspartate type; teichoic acid generally absent; in some strains small amounts of glycerol teichoic acid are detectable. Cell walls do not contain any distinguishing hexoses or pentoses (Cummins and Harris, 1956). Strains appear serologically diverse and no group reactions have been demonstrated.

Acetate or mevalonic acid, riboflavin, calcium pantothenate, niacin and folic acid required. Exogenous thiamine, pyridoxal and thymidine not required. Vitamin $B_{12}$ (cyanocobalamines) generally not required. Mutant strains may require deoxyribosides.