

Genus I. Beijerinckia Derx 1950a, 145^{AL}

CHRISTINA KENNEDY

Beij.e.rinck' i.a. M.L. fem. n. *Beijerinckia* named after M.W. Beijerinck, the Dutch microbiologist (1851–1931).

Straight or slightly curved rods, $\sim 0.5\text{--}1.5 \times 7\text{--}4.5 \mu\text{m}$, with rounded ends. Cells occur singly or appear as dividing pairs. Sometimes large, misshapen cells $3.0 \times 5.0\text{--}6.0 \mu\text{m}$ occur; these are occasionally branched or forked. Intracellular granules of **poly- β -hydroxybutyrate (PHB)** are formed, generally one at each pole. Cysts (enclosing one cell) and capsules (enclosing several cells) may occur in some species. Gram negative. Motile by peritrichous flagella or nonmotile. **Aerobic**, having a strictly respiratory type of metabolism. **N₂ is fixed under aerobic or micro-aerobic conditions**. Optimal temperature for growth, 20–30°C; no growth occurs at 37°C. **Growth occurs between pH 3.0 and pH 9.5–10.0**. **Liquid cultures can become a highly viscous, semitransparent mass**; in some species the whole medium becomes opalescent and turbid, and adhering slime is not produced. **On agar media, especially under N₂-fixing conditions, copious slime is produced** and giant colonies with a smooth, folded, or plicate surface develop; some strains form slime having a more granular consistency. **Catalase positive**. **Glucose, fructose, and sucrose are utilized** by all strains and oxidized to CO₂. No growth occurs on peptone medium. Glutamate is utilized poorly or not at all. Species are found in soils, particularly **tropical regions**.

The mol% G + C of the DNA is: 54.7–60.7.

Type species: ***Beijerinckia indica*** (Starkey and De 1939) Derx 1950a, 146 (*Azotobacter indicum* (sic) Starkey and De 1939, 337.)

FURTHER DESCRIPTIVE INFORMATION

Cell morphology The typical microscopic appearance of *Beijerinckia* cells is shown in Figs. BXII.α.156 and BXII.α.157. Cells may be bicellular due to crosswall formation in the middle of the longitudinal direction of the cell (Figs. BXII.α.158 and



FIGURE BXII.α.157. *Beijerinckia indica* electron micrograph of a thin section showing the two polar lipid bodies, which are surrounded by a membrane ($\times 33,300$).

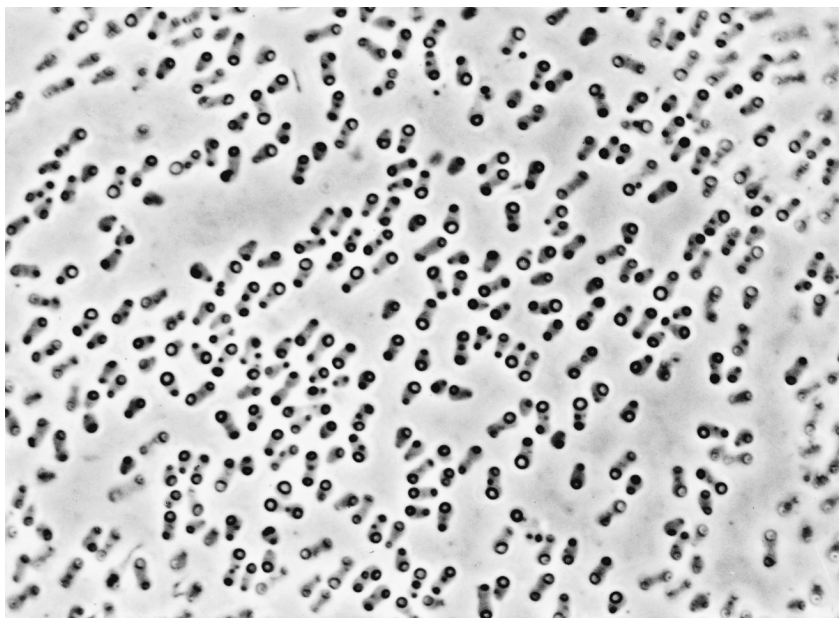


FIGURE BXII.α.156. *Beijerinckia indica* cells cultured on nitrogen-free glucose mineral agar (pH 5.0). The typical appearance of the cells and their intracellular polar lipid bodies is illustrated. Living preparation, phase-contrast microscopy ($\times 1500$).

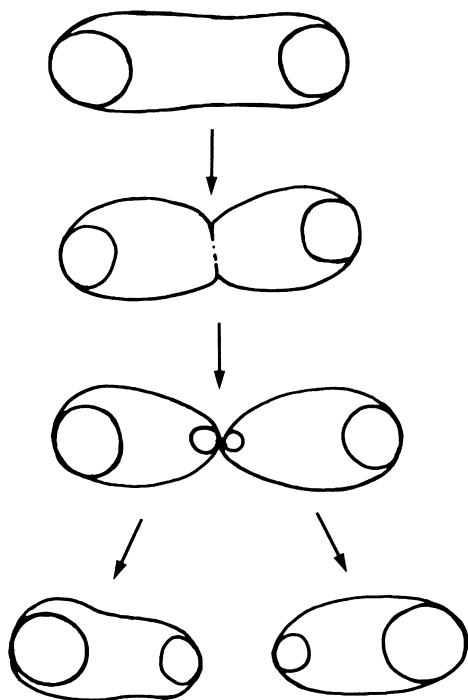


FIGURE BXII.α.158. Diagram of the life cycle of a *Beijerinckia* cell. A cell in division forms a crosswall in the middle of the longitudinal axis of the cell. In actively dividing cells, intermediate stages can often be seen.

BXII.α.159). Under certain cultural conditions, some *Beijerinckia* strains show coccoid cells without terminal lipid globules (Fig. BXII.α.160). Sometimes, especially in *B. mobilis* strains, more than two lipid globules per cell occur (Fig. BXII.α.162). The lipid material is poly-β-hydroxybutyrate (PHB) (Becking, 1974, 1984b). Cyst and capsule formation occur in some species (*B. fluminensis*, *B. mobilis*, and *B. indica*) (Figs. BXII.α.161, BXII.α.162, and BXII.α.163).

The flagella of motile cells are peritrichous in those strains studied (Thompson and Skerman, 1979). Flagella appear to originate from one-half of the often dumbbell-shaped cells. The wave pattern is normal or curly, the wavelength has an average value of ~1.1–1.3 μm, and the amplitude is 0.26–0.35 μm. The amplitude of the waves in *B. fluminensis* and *B. dextrii* strains is usually somewhat larger (0.26–0.35 μm) than in strains of *B. indica* (0.26 μm) (Thompson and Skerman, 1979).

Colonial and cultural characteristics On agar media, especially under N₂-fixing conditions, *Beijerinckia* strains may produce giant colonies containing copious amounts of exopolysaccharide. This slime is often extremely tough, tenacious, or elastic, which makes it difficult to remove part of a colony with a loop. In *B. mobilis* and *B. fluminensis* the exopolysaccharide has a more granular consistency (Becking, 1984a).

On nitrogen-free mineral agar medium¹, *Beijerinckia* species exhibit various kinds of colonial characteristics and pigmentation, and in liquid cultures they show differences in viscosity and pellicle formation; see the individual species descriptions for details.

1. N-free mineral medium, g/l: glucose, 20.0; K₂HPO₄, 0.8; KH₂PO₄, 0.2; MgSO₄·7H₂O, 0.5; FeCl₃·6H₂O, 0.025 or 0.05; Na₂MoO₄·2H₂O, 0.005; CaCl₂, 0.05; and agar, 15.0. The pH is adjusted to 6.9. The CaCl₂ may be omitted to obtain a calcium-free medium.

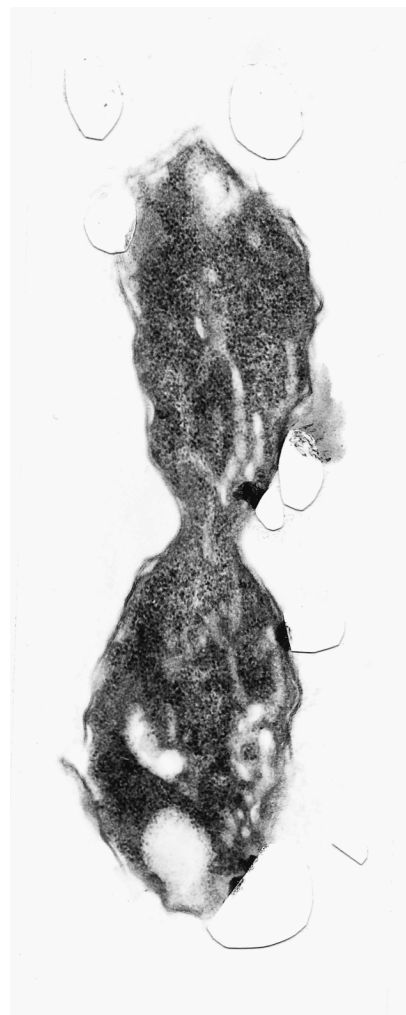


FIGURE BXII.α.159. Electron micrograph of a *Beijerinckia* cell in the process of division. The constriction in the middle of the cell is clearly visible and the two terminal lipid bodies of the original cell can be seen (× 33,000).

The temperature range for growth of *Beijerinckia* species is from 10 to 35°C. Cells are resistant to freezing; no reduction of viability occurs when stored for 3–4 months at –4°C (Becking, 1961).

Metabolism and metabolic pathways Carbon sources utilized include many sugars, organic alcohols, and organic acids (see Table BXII.α.141 and Table BXII.α.142). Since the compilation of strain characteristics by Thompson and Skerman (1979) and Becking (1984a), few reports of new features have appeared. One report describes the ability of *B. mobilis* to utilize several aromatic compounds as carbon sources and as energy sources for nitrogen fixation (Chen et al., 1993); these include benzoate, catechol, 4-hydroxybenzoate, naphthalene, protocatechuate, and 4-toluate. Most *Beijerinckia* species hydrolyze starch.

In alkaline, nitrogen-free, glucose minimal medium, *Beijerinckia* strains decrease the pH to 4.0–5.0. The acids produced are mainly acetic and a small amount of lactic (Kauffmann and Toussaint, 1951; Becking, 1961).

As with other N₂ fixers, *Beijerinckia* species require molybdenum for optimal growth and N₂ fixation. The molybdenum requirement—4.0–35.0 mg/l (Becking, 1962)—is notably higher

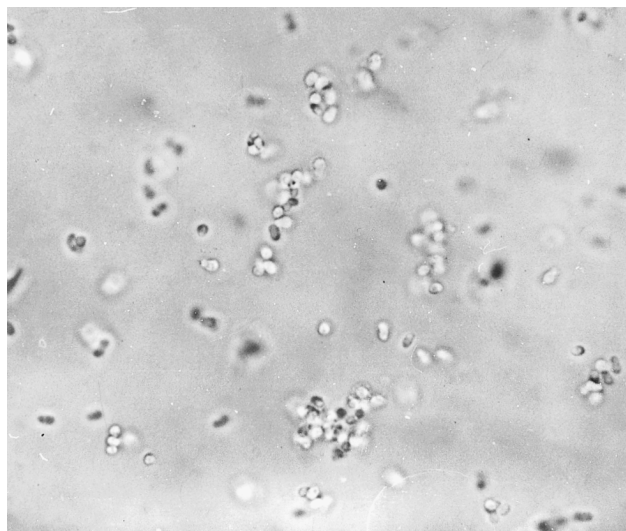


FIGURE BXII.α.160. *Beijerinckia mobilis* from an aged culture. The individual cells often lack the characteristic polar lipid bodies and are more rounded in form, resembling certain *Azotobacter* species ($\times 1000$).

than that of *Azotobacter* and *Azomonas*. Moreover, unlike *Azotobacter*, the molybdenum cannot be replaced by vanadium (Becking, 1962). This finding suggests that alternative nitrogenases are not present in *Beijerinckia*, at least not the nitrogenase that contains vanadium (nitrogenase-2) (for further explanation, see the genus *Azotobacter*, this volume). Growth of *Beijerinckia* species under N_2 -fixing conditions does not require calcium, in contrast to most (but not all) *Azotobacter* species; indeed, $CaCO_3$ is even slightly inhibitory because it tends to prolong the lag phase of growth. The efficiency of N_2 fixation in *Beijerinckia* strains varies from 6 to 17 mg N_2 per g glucose consumed and is inversely proportional to growth (Becking, 1984a); the efficiency is greater at lower carbohydrate and/or oxygen supply levels (Spiff and Odu, 1973; Becking, 1978). The exocellular polysaccharide produced in copious amounts by species of *Beijerinckia* may protect nitrogenase from oxygen damage, as was shown for *B. dextrii* subsp. *venezuelae* (Barbosa and Altherthum, 1992). Removal of the exopolysaccharide resulted in a drastic loss of nitrogenase activity.

Strains of *Beijerinckia indica* and *Beijerinckia mobilis* have been reported to contain large amounts of triterpenoids of the hopane series. In the former species, both bacteriohopanetetrol and aminobacteriohopanetriol were present, whereas in the latter, amino-bacteriohopanetriol was the only C35 hopanoid (Vilcheze et al., 1994). Whether this difference represents a distinguishing feature for all, or several, members of each species was not indicated.

Occurrence and transfer of plasmids No studies involving isolation of mutants or genetic analysis of metabolism have been reported. The broad-host-range IncP plasmid RP4 could be mobilized into *B. indica* by conjugation and stably maintained (Naik et al., 1994). A tributyltin-sensitive strain, *Beijerinckia* sp. MC-27, isolated from freshwater sediment became resistant to this compound after transfer of a plasmid associated with chromium resistance from *Pseudomonas aeruginosa* PAO1 (Miller et al., 1995). The presence of indigenous plasmids in species of *Beijerinckia* was reported (Murai et al., 1990) but no function could be assigned.

Ecology *Beijerinckia* strains were originally isolated from a quartzite soil (pH 4.5) of Malaysia (Altson, 1936) and later from acid soils of Dacca, Bangladesh (pH 4.9), and Insein in Burma (pH 5.2) by Starkey and De (1939). They were later observed to be widely distributed in acidic tropical soils of Africa, Southeast Asia, and South America (Kluyver and Becking, 1955; Becking, 1961). *B. indica* is the most commonly encountered species and has been isolated from tropical soils of all continents and sometimes from nontropical regions. *Beijerinckia* strains can also be recovered from waterlogged soils of wet rice fields (Becking 1961, 1978). In a survey of 392 soils of worldwide distribution, *Beijerinckia* strains were found infrequently in some temperate and subtropical soils (Becking, 1959, 1961; see also the review by Becking, 1981). Nevertheless, their occurrence in temperate habitats might be more widespread than originally thought, based on reports of *Beijerinckia* spp. being isolated from European white fir (Streichan and Schink, 1986). Moreover, acidophilic, methanotrophic organisms with a soluble methane monooxygenase were isolated from three boreal forests in acidic northern wetlands of West Siberia and European north Russia (Dedysh et al., 1998a), and analysis of 16S rDNA sequence from these organisms placed them close to *Beijerinckia indica*. However, more recent studies show that these organisms—unlike *Beijerinckia* species—are unable to grow on sugars and other multicarbon compounds. In addition, *Beijerinckia* cannot grow on methane, and genes coding for methane monooxygenase have not been detected in *B. indica* (Dedysh, personal communication). These bacteria are now named *Methylocella palustris* (Dedysh et al., 2000).

B. mobilis occurs mainly in very acid soils (pH 4.0–4.5) of tropical Southeast Asia, Africa, and South America, and this is in accordance with its ability to fix nitrogen optimally at pH 3.9 (Becking, 1961). This high degree of acid tolerance might be useful for the specific enrichment and isolation of this species. *B. fluminensis* was originally isolated from a “Baixada Fluminensis” (pH 4.2–5.2) of Rio de Janeiro and from some other Brazilian soils (Döbereiner and Ruschel, 1958). It was also isolated from several African and Asian soils (Becking, 1961). *B. dextrii* was originally isolated from an Australian soil (Tchan, 1957) and then found in several South American and Asian locations, as well as in further locations in Australia (Thompson and Skerman, 1979).

In addition to soil or water habitats, *Beijerinckia* species are also found in plant rhizosphere and phyllosphere habitats. Associations with roots of sugarcane in Brazil and with a salt-tolerant grass *Leptochloa fusca* in Pakistan have been reported (Zafar et al., 1987; Baldani et al., 1997). *Beijerinckia* species have also been isolated from the leaves of plants such as coffee, cocoa, and cotton (Ruinen, 1956, 1961; Murty, 1984). There is no evidence that nitrogen fixation by *Beijerinckia* species provides an amount of fixed nitrogen sufficient to benefit plant growth.

ENRICHMENT AND ISOLATION PROCEDURES

An acidic, nitrogen-free medium² can be used for selective isolation of *Beijerinckia* from soil. The low pH of this medium favors development of beijerinckias, which are acid tolerant, and inhibits growth of other organisms, especially *Azotobacter* species. The requirement for trace elements (iron, molybdenum) in this medium is provided by the soil used as the inoculum. The medium is poured as thin layers (2–3 mm deep) into Petri dishes to allow good aeration. This partially inhibits the development

2. Enrichment medium (g/l of distilled water): glucose, 20.0; KH_2PO_4 , 1.0; and $MgSO_4 \cdot 7H_2O$, 0.5. The pH is adjusted to 5.0.

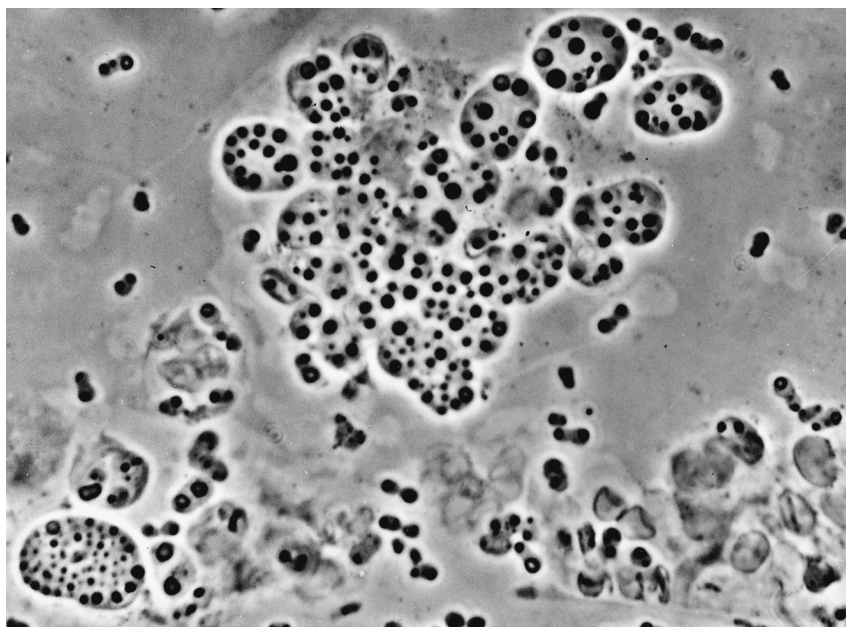


FIGURE BXII.α.161. *Beijerinckia fluminensis* cultured in nitrogen-free glucose mineral agar (pH 5.0), showing distinct capsule formation. The capsules enclose a large number of individual cells. Living preparation, phase-contrast microscopy ($\times 1500$).

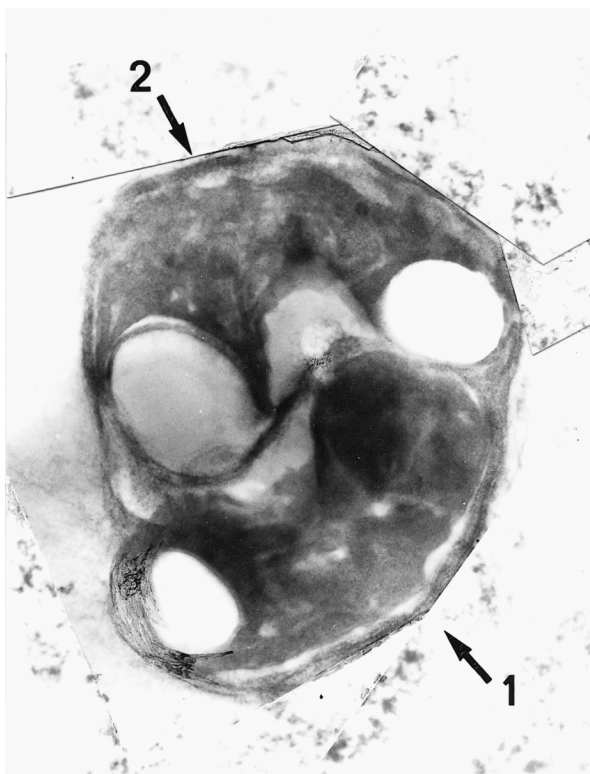


FIGURE BXII.α.162. Electron micrograph of a capsule of *Beijerinckia mobilis* containing two cells (see arrows 1 and 2). The terminal lipoid bodies of each cell are visible and also the distinct capsular wall (see arrow 1) ($\times 33,000$).

or more weeks (*Beijerinckia* strains grow slowly). The entire culture may eventually change into a viscous mass due to slime production. The cultures are examined microscopically at various times for the presence of characteristic *Beijerinckia*-like cells. When such cells occur, the enrichment culture is plated onto a nitrogen-free, mineral agar medium (see Further Descriptive Information). Although an acidic agar medium can be used for isolation, it is not recommended because the agar may be hydrolyzed.

The sieved-soil plate method of Winogradsky (1932), using nitrogen-free mineral agar (pH 4.5–5.0) with glucose or sucrose (10 or 20 g/l), can also be applied. *Beijerinckia* colonies develop after 2–3 weeks around the soil particles on the plates. The type strain of *B. indica* was obtained by this method. In general, however, it is less satisfactory than the use of liquid enrichment media because purification is more difficult (the slime is more tenacious) when one starts with solid media. If the plating method is chosen, a drop inoculation is recommended rather than spreading, because of the higher number of colonies obtained with the former approach (Barbosa et al., 1995).

On agar media, *Beijerinckia* species form characteristic, highly raised, glistening colonies containing a tough, elastic slime. For further purification, a similar medium is used, but it is made neutral or alkaline by the addition of CaCO_3 ³. This is because the slime is more soluble under alkaline conditions, and it is easier to suspend the cells in sterile tap water or liquid medium for further streaking. On the alkaline agar medium, *B. indica* strains usually form highly raised colonies, whereas *B. mobilis* colonies are flatter and produce a uniform reddish brown or amber-brown color on aging.

of anaerobic or facultative N_2 fixers. Approximately 0.5 g of soil, 10 ml of water sample, or detached leaves can be used as the inoculum. The enrichment cultures are incubated at 30°C for 2

3. N-free mineral agar medium with CaCO_3 : composition is similar to that given in Further Descriptive Information, but the CaCl_2 is replaced by CaCO_3 (10–20 g/l).

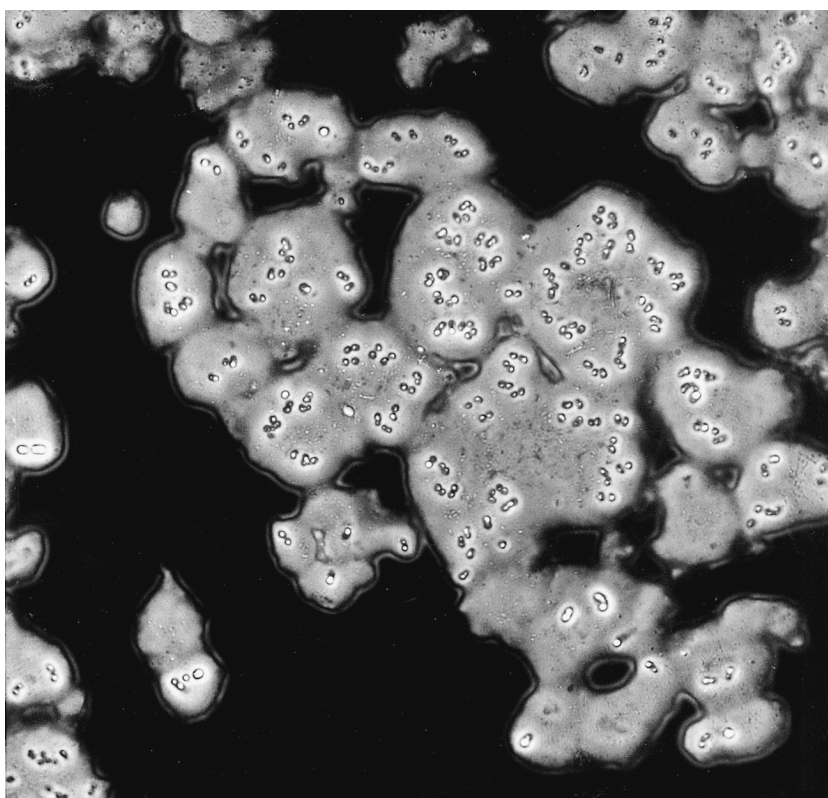


FIGURE BXII.α.163. Cells of *Beijerinckia indica* suspended in India ink, showing the polysaccharide formation around the cells. Living preparation, phase-contrast microscopy. (× 1350)

TABLE BXII.α.141. Differential characteristics of the species of the genus *Beijerinckia* and the genus *Derrxia*^a

Characteristics	<i>B. indica</i>	<i>B. derxii</i>	<i>B. fluminensis</i>	<i>B. mobilis</i>	<i>Derrxia gummosa</i>
Water-soluble, green fluorescent pigment	—	+	—	—	—
Colony color after aging	P	B	P	AB	Br
Motility	[—] ^b	—	[—] ^b	+	+
Resistant to 1% peptone	d	—	—	d	+
Starch hydrolyzed	—	d	—	—	—
Growth on asparagine as C and N source	—	—	—	d	+
Urea hydrolyzed	+	+	—	+	+
H ₂ S production from cysteine	—	—	+	—	—
Tween 20 hydrolyzed	—	—	—	—	+
Indole produced	—	—	—	—	+
Antagonism to Gram positive organisms	—	d	—	—	+

^aFor symbols, see standard definitions; P, pink; B, buff; AB, amber-brown; Br, brown. For additional distinguishing characteristics, see Table BXII.α.142, utilization of carbon compounds.

^bIf positive, motility occurs mostly in young stages and the cells are usually only weakly motile.

Specific enrichment procedures No specific procedure is known to select for a particular *Beijerinckia* species, and all existing strains are random isolates obtained from soil using one of the general procedures outlined above. In general, carbon source utilization is not distinctive for particular species, although certain substrates might be useful for enrichment of one or two particular species because of their preferential use: e.g., benzoate for enrichment of *B. mobilis* and mannose for *B. indica* or *B. fluminensis* (see Table BXII.α.142). Thompson and Skerman (1979) suggested that certain substrates or inhibitors might be useful for enrichment of various species, although this has not yet been experimentally tested. From the properties of the strains

studied, the following compounds have potential value for enrichment or selection:

1. *B. indica*: L-arabinose, D-mannose, glycerol, caprylate, and *trans*-aconitate. Glycerol might be useful for *B. indica*, and for *Derrxia gummosa* and some *B. fluminensis* strains. Nitrilotriacetate might also be useful for *B. indica*.
2. *B. mobilis*: pentan-2-ol, 1,3-butylene glycol, asparagine, and *n*-valerate might be useful. Phenol (0.05%) might be used to inhibit the growth of other *Beijerinckia* species.
3. *B. indica* and *B. mobilis*: propan-1-ol, butan-1-ol, or 1,3-propylene glycol for enrichment.

4. *B. indica* subsp. *lacticogenes* and *B. mobilis*: caproate, *p*-hydroxybenzoate, or phenol for enrichment.
5. *B. derxii* and *B. fluminensis*: α -methyl-D-glucoside, maltose, melibiose, or melezitose for enrichment. Where both species are present, *B. derxii* would likely outgrow the extremely slow-growing *B. fluminensis*.
6. *B. derxii* subsp. *venezuelae*: L-arabitol for enrichment.

Because the colony morphology and chromogenesis of growth on solid media differs among the various *Beijerinckia* species, primary selection of colonies from the enrichment cultures is done mainly on the basis of these colonial characteristics. A more precise identification is performed later using additional differential characteristics.

MAINTENANCE PROCEDURES

Beijerinckia strains can be lyophilized in skim milk or dextran-sodium glutamate solution on filter paper and stored in the dark at room temperature (Becking, 1984a).

Storage has also been achieved on the usual agar media in tubes plugged with sterile rubber seals, with storage in the dark at room temperature (Antheunisse, 1972, 1973); after 10 years, 33% of the cultures retained viability. *Beijerinckia* cultures stored under a seal of sterile liquid paraffin or mineral oil generally survive for at least 3–5 years (Becking, 1984a).

Strains may also be preserved indefinitely in liquid nitrogen. At the type culture collection in Delft, The Netherlands, DMSO (10%, v/v) is added to cultures in the log phase or end of log phase, and the cultures are frozen as rapidly as possible in liquid nitrogen. For recovery, the vials are thawed rapidly in a waterbath at 37°C (Becking, 1984a).

Cryopreservation in 10% glycerol was also successful (Thompson, 1987). See the genus *Azotobacter* for details.

DIFFERENTIATION OF THE GENUS *BEIJERINCKIA* FROM OTHER GENERA

Beijerinckia strains can be distinguished from other aerobic N₂ fixers by their great acid tolerance (which allows them to grow well at pH 4.0 or 5.0), by their failure to form a pellicle on the surface of liquid media, and by their ability to make a liquid medium viscous by slime production (Fig. BXII.α.164). Moreover, on solid media they produce characteristic large, slimy colonies having a tough, tenacious, and sometimes elastic slime.

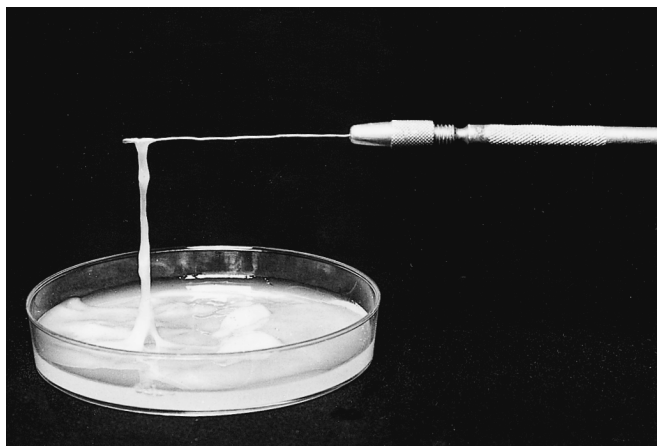


FIGURE BXII.α.164. Enrichment culture of *Beijerinckia* inoculated with tropical soil, demonstrating the highly viscous consistency of the medium after 3 weeks ($\times 0.8$).

Because of this exopolysaccharide production, it is often difficult to subculture portions of a colony for purification. For other features distinguishing *Beijerinckia* strains from *Derrxia**, the most closely related genus, see Tables BXII.α.141 and BXII.α.142.

Beijerinckia cells can be distinguished from those of *Azotobacter* and *Azomonas* by their generally smaller size, by their more rod-shaped or sometimes pear- or dumbbell-shaped appearance, and by the characteristic presence of a lipid body at each pole (Fig. BXII.α.156). Many strains of *Beijerinckia* utilize nitrate poorly or not at all and, in this respect, differ from strains of *Azotobacter*.

Although both *Beijerinckia* and *Derrxia* produce slimy colonies on agar and viscosity in broth, *Beijerinckia* strains can be distinguished by (a) failure to produce dark mahogany-brown colonies with aging, (b) cells that contain bipolar lipid bodies rather than numerous lipid bodies throughout the whole cell, (c) failure to form a pellicle at the surface of liquid media, and (d) a positive catalase reaction. In addition, a number of C sources should distinguish the two genera; for instance, *Beijerinckia* species, but not *Derrxia*, can utilize sorbose and raffinose, and *Derrxia*, but not *Beijerinckia*, utilizes aspartate, glutamate, or ethylamine (Table BXII.α.142).

TAXONOMIC COMMENTS

When numerical analysis methods are applied to species of *Beijerinckia*, *Azotobacter*, and *Azomonas*, the *Beijerinckia* species fuse into a single, apparently coherent group (Thompson and Skerman, 1979). Using a wide range of attributes, and considering all strains of named and unnamed *Beijerinckia* species, numerical analysis supports the concept of a separate genus for these bacteria. In addition, the experiments reported by De Smedt et al. (1980), in which ¹⁴C-labeled rRNA from *Beijerinckia indica* was hybridized with filter-fixed DNA from a wide variety of Gram-negative bacteria, indicated the genus *Beijerinckia* to be a heterogeneous but coherent group. From rRNA cistron similarities, it was concluded that *Beijerinckia* and *Azotobacter*/*Azomonas* belong to different classes. Analysis of the 16S rDNA sequence of *Beijerinckia indica* places this genus within the phylum *Proteobacteria*. The family *Beijerinckiaceae*, including the genera *Beijerinckia*, *Derrxia*, and *Chelatococcus*, is in the class *Alphaproteobacteria* and the order *Rhizobiales*. This family is most closely related to the families *Methylocystaceae* and *Bradyrhizobiaceae*.

Thompson and Skerman (1979) used many phenotypic characters of *Beijerinckia* strains for numerical analysis, yielding a hierarchical classification. These authors confirmed the presence of four species, in two main groups within the genus. Group 1294 (*B. fluminensis* + *B. derxii*) fuses with group 1289 (*B. indica* subsp. *indica* + *B. indica* subsp. *lacticogenes* + *B. mobilis*) to produce the group 1297. Group 1289 strains generally differed from group 1294 strains in having thinner cells and using caprylate, propan-1-ol, 1,3-propylene glycol, *trans*-aconitate, nitrilotriacetate, and L-arabinose, but not maltose and α -methyl-D-glycoside, as sole carbon sources. Moreover, strains of group 1289 produced acid from L-arabinose and glycerol and were resistant to 0.5% NaCl, 0.05% phenol, and 1.0% sodium benzoate. One widely studied member of the genus, *Beijerinckia* sp. strain B1, was reclassified as a strain of *Sphingomonas yanoikuyae* on the basis of 16S rDNA analysis (Khan et al., 1996a).

*Editorial Note: Because of the 16S rDNA placement, the genus *Derrxia* has been moved to the family *Alcaligenaceae* and is no longer listed in *Beijerinckiaceae*.

TABLE BXII.α.142. Utilization of carbon compounds by *Beijerinckia* and *Dexia* species^a

Carbon compounds utilized ^{b,c}	<i>B. indica</i> (14) ^d	<i>B. derxii</i> (21)	<i>B. fluminensis</i> (10)	<i>B. mobilis</i> (2)	<i>Dexia gummosa</i> (6)
Arabinose	+	—	d	+	—
Galactose	+	+	+	—	—
Fructose	+	+	+	—	+
Melibiose	d	d	+	—	—
Maltose	—	+	d	—	—
Mannose	+	—	d	—	—
Sorbose	+	d	+	+	—
Raffinose	+	+	d	d	—
Xylose	—	—	+	—	—
Butanol	+	—	d	+	+
Propanol	+	—	—	+	+
Glycerol	+	—	+	+	+
Sorbitol	+	+	+	—	d
Mannitol	+	+	d	d	d
Acetate	d	+	—	+	+
Citrate	+	d	—	d	+
Oxaloacetate	+	d	—	—	+
Fumarate	+	d	d	d	+
Malate	+	d	—	d	—
Malonate	—	—	—	—	+
Glycolate	+	+	—	+	+
Benzoate	—	—	—	+	—
L-Ascorbate	+	d	+	—	—
Aspartate	—	—	—	—	+
Glutamate	—	—	—	—	+
Ethylamine	—	—	—	—	+

^aFor symbols, see standard definitions.^bData represent a merger of information in Thompson and Skerman (1979) and Becking (1984a) in the 1st edition of *Bergey's Manual of Systematic Bacteriology*. Not all compounds tested by Thompson and Skerman (1979) are included.^cAll species can utilize glucose, sucrose, lactate, pyruvate, succinate, gluconate, and fumarate. All species fail to utilize ribose, fucose, cellobiose, lactose, trehalose, glutarate, and oxoglutarate.^dNumbers in parentheses represent the number of strains tested.

ACKNOWLEDGMENTS

The description of the genus as given by J.-H. Becking in the 1st edition of the *Manual* remains largely unchanged; information has been reorganized, reevaluated, and updated.

FURTHER READING

Becking, J.H. 1992. The Genus *Beijerinckia*. In Balows (Editor), *The Prokaryotes. A Handbook on the Biology Of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd Ed., Vol. 3, Springer-Verlag, New York. pp. 2254–2267.

List of species of the genus *Beijerinckia*

1. ***Beijerinckia indica*** (Starkey and De 1939) Derx 1950a, 146^{AL} (*Azotobacter indicum* (sic) Starkey and De 1939, 337.) *in' di.ca.* L. fem. adj. *indica* of India.

The characteristics are as described for the genus and as given in Tables BXII.α.141 and BXII.α.142, with the following additional information. Straight or slightly curved rods 0.5–1.2 × 1.6–3.0 μm. PHB granules persist in aged cultures. No resting stages occur; neither cyst nor spore formation is observed.

Agar colonies are raised. At first, they are semitransparent but soon become uniformly turbid or opaque white. On aging, the colonies develop a light reddish pink, cinnamon, or fawn color on neutral or alkaline media; on acid media, they remain colorless. On acid media, the slime is more tenacious, tough, and elastic than on alkaline media. Giant colonies may develop, first with a smooth surface (Fig. BXII.α.165), but later with a folded or wrinkled surface (Fig. BXII.α.166).

Liquid media become viscous as cell density increases.

Color may be produced, but is less prominent than on agar.

Grows between pH 3.0 and 10.0 (optimum is 4.0–10.0). Temperature range for growth, 10–35°C; no growth at 37°C.

Growth on, and utilization of, nitrate is poor, and N₂ is fixed in preference to utilization of nitrate in the medium (Becking, 1962). Weak growth occurs on malt agar, no growth in plain broth or peptone agar.

Widely distributed in acid tropical soils.

The mol% G + C of the DNA is: 54.7–58.5 (*T_m*) (De Ley and Park, 1966; De Smedt et al., 1980).

Type strain: Delft E.II.12.1.1, ATCC 9039, DSM 1715, NCIB 8712, WR-119.

GenBank accession number (16S rRNA): M59060.

Derx (1950a) described “*B. indica* biovar *alba*”, which was distinguished by its lack of pigmentation on aging; however, under extreme (alkaline) conditions it produced a pink pigment. In the hierarchical classification of Thompson and Skerman (1979), one co-type (strain WR-236) of *B. indica* biovar *alba* was placed in *B. indica* subsp. *lacti-*

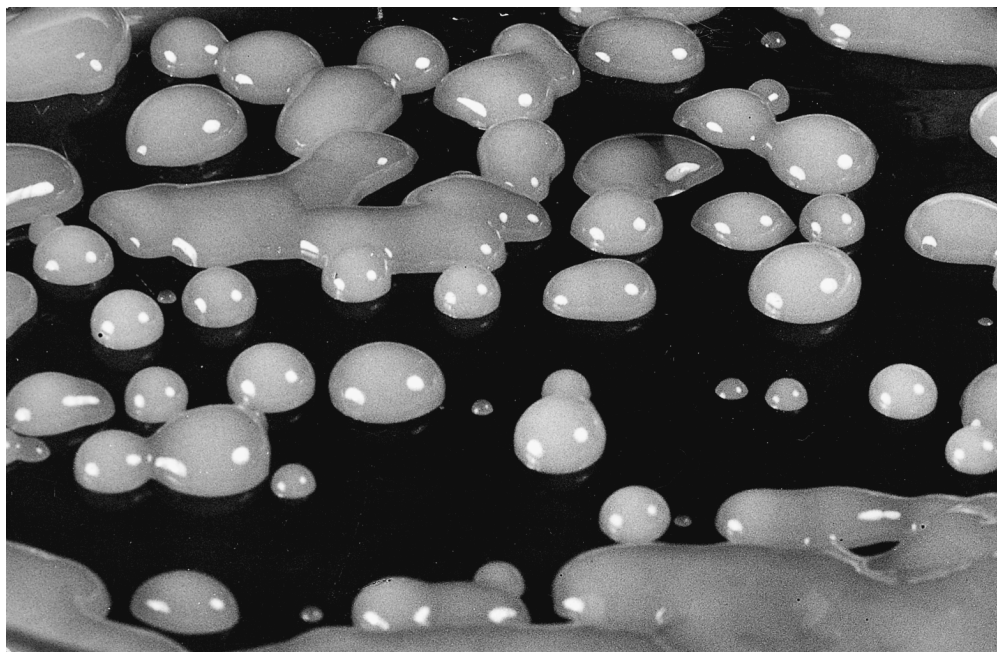


FIGURE BXII.α.165. Typical colony type of *Beijerinckia indica* on nitrogen-free glucose mineral agar. The colonies are highly raised and have a very tough, elastic slime. In young cultures these colonies are colorless and transparent ($\times 2$).



FIGURE BXII.α.166. Typical colony of an aged culture of *Beijerinckia indica* on nitrogen-free glucose mineral agar. The colonies increase greatly in size due to copious slime production. The colonies become massive and opaque, with a plicate surface. In this stage they often attain a light reddish, pink, or cinnamon color, especially on neutral or alkaline media ($\times 2$).

cogenes (group 1270) and the other (strain WR-235) was placed in *B. dextrii* subsp. *venezuelae* (group 1271).

- a. ***Beijerinckia indica* subsp. *indica*** (Starkey and De 1939) Derx 1950a, 146^{AL} (*Azotobacter indicum* (sic) Starkey and De 1939, 337.)

Thompson and Skerman (1979) distinguished *B. indica* subsp. *indica* from subsp. *lacticogenes* by differences in organic carbon utilization, nitrate reduction, and resistance to peptone-nitrogen. Based on their study of nine strains of subsp. *indica* and four or five strains of

subsp. *lacticogenes*, the only absolute character that differentiated the two subspecies was the failure of subsp. *indica* to utilize *p*-hydroxybenzoate as a sole carbon source. Nitrate was reduced to nitrite by eight of nine strains of subsp. *indica* but not by four of four strains of subsp. *lacticogenes*. The differences in utilization of sole carbon sources by subsp. *indica* vs. subsp. *lacticogenes* were as follows: propan-2-ol, 7/9 vs. 5/5; butan-2-ol, 0/9 vs. 3/5; D-arabitol, 4/9 vs. 1/5; phenol, 0/9 vs. 4/5; caproate, 0/9 vs. 4/5; adipate, pimelate, suberate, azelate, and sebacate, 0/9 vs. 1/5 or 1/4; α -oxybutyrate, 0/9 vs. 1/5; fumarate, DL-malate, tartrate (D, L, and *meso*), oxaloacetate, mucate, and *trans*-aconitate, 9/9 vs. 3–4/5. None of the strains of subsp. *indica* lacked flagella, whereas half the strains of subsp. *lacticogenes* did lack flagella.

The mol% G + C of the DNA is: 54.7–58.5 (T_m) (De Ley and Park, 1966; De Smedt et al., 1980).

Type strain: Delft E.II.12.1.1, ATCC 9039, DSM 1715, NCIB 8712, WR-119.

GenBank accession number (16S rRNA): M59060.

- b. *Beijerinckia indica* subsp. *lacticogenes* (Kauffmann and Toussaint 1951) Thompson and Skerman 1981, 215^{VP} (Effective publication: Thompson and Skerman 1979, 332) (*Azotobacter lacticogenes* Kauffmann and Toussaint 1951, 710)

lac.ti.co.ge.nes. M.L. n. *acidum lacticum* lactic acid; Gr. v. *gennaio* to produce; M.L. adj. *lacticogenes* lactic acid producing (which is an error: acetic acid is produced).

This subspecies is distinguished from *B. indica* subsp. *indica* by the characteristics described above, and by the consistency of the colonies (less elastic and rubbery (cartilaginous) and more butyrous and brittle than those of subsp. *indica*). Moreover, subsp. *lacticogenes* is somewhat less resistant to diamond fuchsin, brilliant green, sodium fluoride, and streptomycin. In contrast to subsp. *indica*, four of five strains of subsp. *lacticogenes* grown on agar containing *p*-hydroxybenzoate could metabolize protocatechuate via the *ortho*-cleavage pathway (Thompson and Skerman, 1979).

The mol% G + C of the DNA is: unknown.

Type strain: WR-119, ATCC 19361.

2. *Beijerinckia derxii* Tchan 1957, 315^{AL}

derx'i.i. M.L. gen. n. *derxii* of Derx; named after H.G. Derx, the Dutch microbiologist (1894–1953).

The characteristics are as described for the genus and as given in Tables BXII.α.141 and BXII.α.142, with the following additional information. Single straight or curved rods, or rods with clavate extremities, 1.5–2.0 × 3.5–4.5 μm. Polar lipid bodies are very large and conspicuous. No cyst or capsule formation occurs. Nonmotile.

Colonies are highly raised, slimy, and smooth. The chemical composition of the slime has not yet been examined. Colonies are at first semitransparent or opaque white, but after 2–3 weeks, a yellow-green, water-soluble, fluorescent pigment is produced, particularly on iron-deficient media. When the pigment first appears it remains within the colony, but later it diffuses into the agar medium. Under certain conditions, pigment production on agar media may be very poor or absent.

Liquid cultures become uniformly turbid and pigment production is usually less than on solid media.

Growth occurs between pH 4.0 and 9.0 (optimum, 6.0–7.0). There is no growth at pH 3.0 or 11.0. Temperature range for growth, 10–35°C; no growth at 37°C.

Isolated from soils from Queensland, Northern Australia, and neutral and alkaline soils of South Africa.

The mol% G + C of the DNA is: 59.1 ± 1.6 (T_m) (De Ley and Park, 1966).

Type strain: Q13 of Tchan, ATCC 49361, DSM 2328, UQM 1968.

- a. *Beijerinckia derxii* subsp. *derxii* Tchan 1957, 315^{AL}

In the hierarchical classification of Thompson and Skerman (1979), the strains within the species *B. derxii* can be divided into two main groups: group 1263 and group 1271. Group 1263 contains a co-type of *B. derxii* Tchan 1957 and three co-type strains of "*Beijerinckia congensis*" Hilger 1965, as well as three Australian isolates. This group was named by Thompson and Skerman (1979) as *B. derxii* subsp. *derxii*. Differences between this subspecies and *B. derxii* subsp. *venezuelae* (group 1271) are not markedly consistent, apart from utilization of nitrate, as outlined below in the description of the subsp. *venezuelae*.

The mol% G + C of the DNA is: not available.

Type strain: Q13 of Tchan, ATCC 49361, DSM 2328, UQM 1968.

- b. *Beijerinckia derxii* subsp. *venezuelae* (Materassi, Florenzano, Balloni and Favilli 1966) Thompson and Skerman 1981, 215^{VP} (Effective publication: Thompson and Skerman 1979, 343) (*Beijerinckia venezuelae* Materassi, Florenzano, Balloni and Favilli 1966, 210.)

ven.e.zue'lae. M.L. gen. n. *venezuelae* of Venezuela, South America.

In the hierarchical classification of Thompson and Skerman (1979), group 1271 is classified as *B. derxii* subsp. *venezuelae*. This group consists of six co-type strains of "*B. venezuelae*" Materassi et al. 1966, one co-type of "*B. indica* biovar alba" Derx 1950a, and three Australian isolates.

Differences between group 1263 (subsp. *derxii*) and group 1271 (subsp. *venezuelae*) are not markedly consistent. In general, strains of group 1271 are distinguished by not utilizing nitrate as a sole source of nitrogen, being nonmotile, not hydrolyzing glycogen, growing over a slightly wider pH range (not more than 0.5 pH unit at each end of the range), and by differences in utilization of several organic compounds as sole sources of carbon. Of 14 strains of subsp. *venezuelae* and 7 strains of subsp. *derxii* tested by Thompson and Skerman (1979), the utilization of these substrates was as follows: melibiose, 5/14 vs. 7/7; propan-2-ol, 6/14 vs. 0/7; L-arabitol, 6/14 vs. 0/7; propionate, 7/14 vs. 0/7; fumarate, 8/14 vs. 1/7; and L-ascorbate, 6/14 vs. 5/7. Eleven of 14 strains of subsp. *venezuelae* used nitrate as a nitrogen source, whereas only 1 of 7 strains of subsp. *derxii* could do so.

The mol% G + C of the DNA is: not available.

Type strain: 2 of Materassi, DSM 2329, WR-222.

Of the six strains of subsp. *venezuelae* provided by G. Florenzano (i.e., strains WR-221 to WR-226), it is not

clear how many independent isolates are represented (Becking, 1984a).

3. *Beijerinckia fluminensis* Döbereiner and Ruschel 1958, 269^{AL}

flu.mi.nen'sis. M.L. adj. *fluminensis* named after the locality "Baixada Fluminense", State Rio de Janeiro, Brazil, from which soil it was first isolated.

The characteristics are as described for the genus and as given in Tables BXII.α.141 and BXII.α.142, with the following additional information. Straight or slightly curved rods, $1.0\text{--}1.5 \times 3.0\text{--}3.5 \mu\text{m}$. Older cultures show characteristic large capsules enclosing 2–10 or more individual cells. Division of cells within the capsules has been observed. Motility is slow or absent, especially in older cells.

Colonies are typically small and granular, moderately raised, with an irregular rough surface (Fig. BXII.α.167). The slime is not liquid, tenacious, or elastic, but more granular and stiff; its chemical composition has not yet been examined. Colonies are at first opaque white, becoming pink, reddish brown, or fulvous (like *B. indica*) after 1–2 weeks on neutral or alkaline media.

Slime production in liquid media is reduced. No pellicle or viscosity occurs, but a bluish white turbidity develops.

Grows between pH 3.5 and 9.2. Temperature range for growth, 10–35°C (optimum, 26–33°C); no growth at 37°C.

Found in acidic soils of South America, Africa, and Asia (China, Indonesia).

The mol% G + C of the DNA is: 56.2 ± 1.8 (T_m) (type strain) (De Ley and Park, 1966; De Smedt et al., 1980).

Type strain: CD10 of Döbereiner and Ruschel, DSM 2327, UQM 1685.

4. *Beijerinckia mobilis* Derx 1950b, 10^{AL} (*Beijerinckia mobile* (sic) Derx 1950b, 10.)

mo'bi.lis. L. fem. adj. *mobilis* movable, motile.

The characteristics are as described for the genus and as given in Tables BXII.α.141 and BXII.α.142, with the following additional information. Straight, curved, or pear-

shaped rods, $0.6\text{--}1.0 \times 1.6\text{--}3.0 \mu\text{m}$. Misshaped or forked cells sometimes occur. "Ascococcus"-like clusters of cells are often visible in older cultures. The typical polar lipid bodies may disappear in aging cells, and the cells are then more rounded and resemble *Azotobacter* cells (Fig. BXII.α.160). Motility is conspicuous.

Agar colonies are not as raised as those of *B. indica*, and slime production is less (Fig. BXII.α.168). The slime is neither elastic nor sticky; its chemical composition has not yet been examined. Older cultures on neutral or alkaline agar media show a typical dark amber or deep reddish brown color.

Broth cultures do not become viscous. There is a tendency to form a pellicle at the surface.

Grows between pH 3.0 and 10.0. Optimal growth and N₂ fixation occur at pH 4.0–5.0 and decrease sharply at the more alkaline values (Becking, 1961). Temperature range for growth, 10–35°C; no growth at 37°C.

All strains tested have grown well on nitrate or ammonium salts as the nitrogen source (in contrast to *B. indica*). Weak growth or no growth occurs on urea, glycine, glutamate, or tyrosine. All strains grow on leucine and casein agar. Moderate growth occurs on malt agar. The differences in levan production from sucrose observed by Derx (1950b) are variable and cannot be used for differentiation of this species.

Common in Indonesian (Java) soils; also isolated from soils of South America (Surinam) and tropical Africa.

The mol% G + C of the DNA is: 57.3 (T_m) (De Smedt et al., 1980).

Type strain: Delft E.III.12.2, ATCC 35011, DSM 2326, UQM 1969.

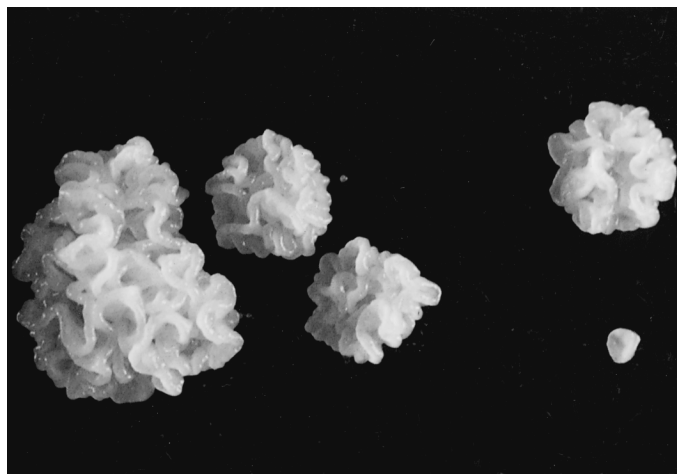


FIGURE BXII.α.167. Typical colonies of *Beijerinckia fluminensis* on nitrogen-free glucose mineral agar. This species forms rather small, raised colonies with a highly plicate surface. In this species the slime has a granular consistency ($\times 4$).

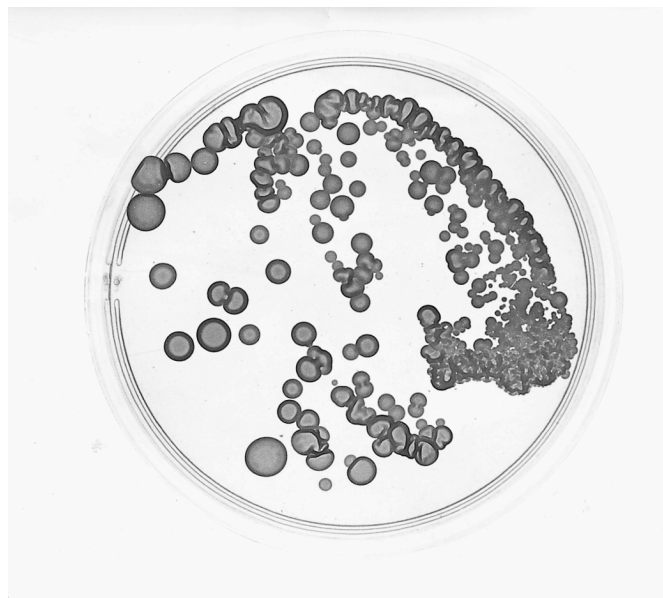


FIGURE BXII.α.168. Colonies of *Beijerinckia mobilis* on a nitrogen-free glucose mineral agar containing CaCl₂. On this transparent medium, the species forms only small raised colonies having a typical amber-brown color on aging ($\times 0.7$).

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