8 The Family *Cohaesibacteraceae*: The Genera *Cohaesibacter* and *Breoghania*

Cintia P. J. Rua[1] · *Fabiano Thompson*[2]
[1]Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil
[2]Laboratory of Microbiology, Institute of Biology, Center for Health Sciences, Federal University of Rio de Janeiro (UFRJ), Ilha do Fundaõ, Rio de Janeiro, Brazil

Abstract
This chapter reviews the recently described family *Cohaesibacteraceae*, their two genera (*Cohaesibacter* and *Breoghania*) and three species (*Cohaesibacter gelatinilyticus* Hwang and Cho 2008, *Cohaesibacter marisflavi* Qu et al. 2011, and *Breoghania corrubedonensis* Gallego et al. 2010). The type species for each genus are *Cohaesibacter gelatinilyticus* and *Breoghania corrubedonensis*, respectively. *Cohaesibacteraceae* was created to accommodate strains that were not genetically similar enough to be classified into existing families of the order *Rhizobiales*. Besides the genetic dissimilarity, the sole major respiratory quinone (Q-10) and the polar lipid composition differentiate *Cohaesibacteraceae* from the other families of this order.

The members of *Cohaesibacteraceae* are Gram-negative, facultative anaerobic (*Cohaesibacter*) or aerobic (*Breoghania*) rods and are motile by polar flagella. *Cohaesibacter* comprises strains isolated from coastal waters of the east coast of Korea and China, whereas *Breoghania* has its type strain isolated from coastal waters of northwest Spain. Nothing is known about their applications or ecological importance, so this chapter only provides phenotypic and genetic characterization. Additionally, the phylogenetic relationship with other families of the order *Rhizobiales* is presented. Finally, the formal taxonomical descriptions of both genera and their respective species are given.

Introduction and General Characteristics

The family *Cohaesibacteraceae* is in the order *Rhizobiales*, which is one of the seven orders of Alphaproteobacteria. *Cohaesibacteraceae* is distinguished from the other 12 families of Rhizobiales (*Rhizobiaceae*, *Bartonellaceae*, *Brucellaceae*, *Phyllobacteriaceae*, *Methylocystaceae*, *Beijerinckiaceae*, *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, *Methylobacteriaceae*, *Rhodobiaceae*, *Aurantimonadaceae*, and *Xanthobacteraceae*) by phylogenetic analysis of ribosomal DNA (16S). This recently proposed family includes two genera: *Cohaesibacter* and *Breoghania*. They are distinct lineages formed by strains from coastal marine environments that did not cluster with any other families in 16S phylogenetic analysis. In addition, the members of *Cohaesibacteraceae* have other genetic and phenotypic characteristics, which are discussed in detail in this chapter. The family and the type species of the genus *Cohaesibacter*, *Cohaesibacter gelatinilyticus*, had their emended description approved by the International Committee on Systematics of Prokaryotes and its judicial commission (Euzéby 2011).

Phylogeny

Phylogenetic analyses, including secondary structure information of 16S rRNA, from 189 species of the order *Rhizobiales* determined that strains from coastal seawaters of the east coast of Korea (CL-GR15 and CL-GR35) belong to that order but do not represent any previously described family. Thus, the family *Cohaesibacteraceae* was characterized. Later, another seawater strain (DQHS21) from the east coast of China showed greater similarity with 16S of *Cohaesibacter gelatinilyticus* Hwang and Cho 2008 than with the other Alphaproteobacteria genera. Hwang and Cho (2008) detected three signature nucleotides in the 16S gene that characterize the genus *Cohaesibacter* in a study describing the type species *Cohaesibacter gelatinilyticus*. In addition, a strain (UBF-B1) isolated from a beach in the northwest of Spain after an oil spill was phylogenetic compared with *Rhizobiales* sequences (including *Cohaesibacteraceae* strains) using the 16S fragment and other genes (atpD, pyrG, rpo and fusA). Results showed UBF-B1 clustering together with *Cohaesibacter gelatinilyticus*; however, they had only 92 % similarity. These data resulted in a new genus description: *Breoghania*.

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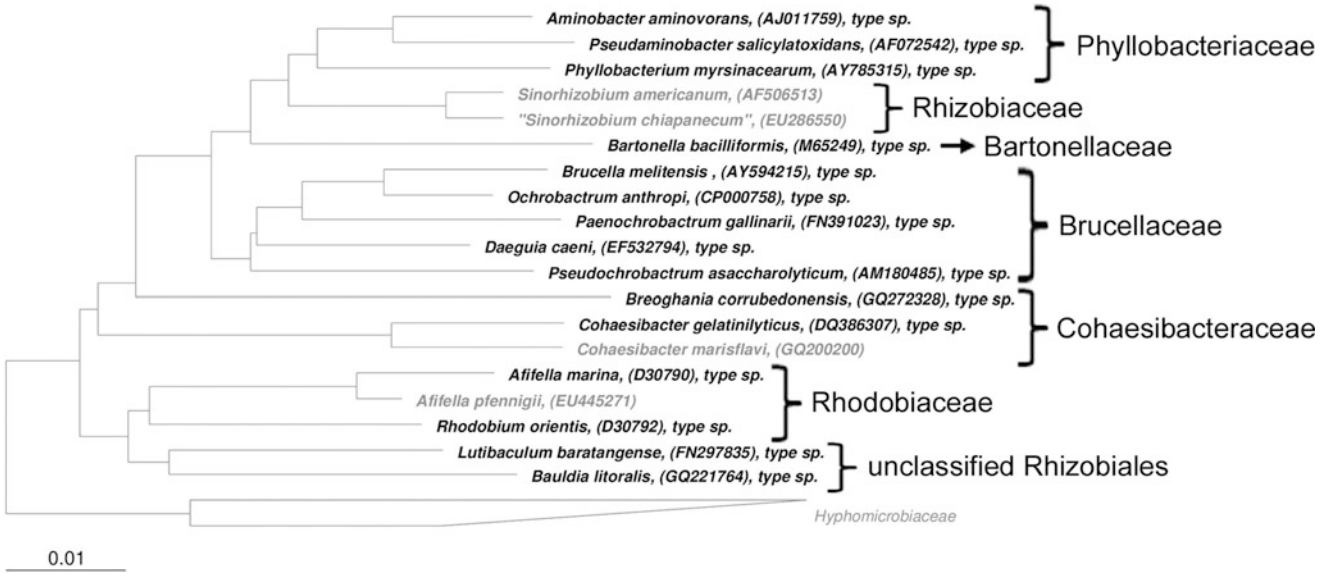

Fig. 8.1

Phylogenetic position of the family *Cohaesibacteraceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with a representative set of nearly 750 high-quality type-strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Taxonomy

Family *Cohaesibacteraceae*, Hwang and Cho (2008) emend. Gallego et al. (2010)

Cohaesibacteraceae (Co.hae.si.bact.er.a'ce.ae. N.L. masc. n. *Cohaesibacter*, type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Cohaesibacteraceae*, the Cohaesibacter family) (Hwang and Cho 2008).

Cohaesibacteraceae is a family phylogenetically positioned in the order *Rhizobiales* in the class *Alphaproteobacteria*. It is most closely related with the families *Brucellaceae* (genera *Ochrobactrum* and *Brucella*: 90.9–92.5 % similarity); *Rhizobiaceae* (genus *Sinorhizobium*: 90.9–91.5 %); *Bartonellaceae* (genus *Bartonella*: 90.6–91.9 %); *Rhodobiaceae* (genus *Rhodobium*: 89.3–91.2 %), and *Phyllobacteriaceae* (genus *Phyllobacterium*: 89.3–91.2 %) (◉ *Fig. 8.1*).

The members of the family have varying capabilities for nitrate reduction and the DNA composition ranges from 52 to 64 mol% G+C. The sole major respiratory quinone (Q-10) differentiates *Cohaesibacteraceae* from the families *Aurantimonadaceae*, *Phyllobacteriaceae*, *Rhodobiaceae*, *Bradyrhizobiaceae*, *Methylocystaceae* and *Hyphomicrobiaceae*. *Cohaesibacteraceae* is also differentiated from 10 families in the order *Rhizobiales* (except the families *Brucellaceae* and *Phyllobacteriaceae*) by polar lipid composition (◉ *Table 8.1*).

Genus *Cohaesibacter*, Hwang and Cho (2008) emend. Gallego et al. (2010)

Cohaesibacter [Co.hae.s'i.bac.ter. L. part. adj. *cohaesus* (from L. v. *cohaereo*) pressed together, clung together; N.L. masc. n. bacter a rod; N.L. masc. n. *Cohaesibacter* rods that appear cohesive with each other].

The members of this genus are Gram-negative, facultative anaerobic rods. They have oxidase activity and variable catalase activity. The predominant fatty acids are $C_{18:1}\omega7c$ and $C_{15:0}$ iso 2-OH and/or $C_{16:1}\omega7c$ and $C_{18:0}$. The respiratory quinone is ubiquinone 10 (Q-10). The DNA G+C content ranges from 53.0 to 55.2 mol%. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, an unidentified aminolipid (AL1) and an unidentified glycolipid. The species of the genus have genetic signatures that are two compensatory transversion mutations (positions 678: A and 712: T) and a single transversion mutation (position 194: T). The type species is *Cohaesibacter gelatinilyticus*.

Description of *Cohaesibacter gelatinilyticus*, Hwang and Cho (2008)

Cohaesibacter gelatinilyticus (ge.la.ti.ni.ly'ti.cus. N.L. n. *gelatinum* gelatin; Gr. adj. *lutikos* able to dissolve; N.L. adj. *lyticus* dissolving; N.L. masc. adj. *gelatinilyticus* gelatin-dissolving).

Table 8.1

Families: *1 Cohaesibacteraceae* fam. nov., *2 Brucellaceae*, *3 Bartonellaceae*, *4 Aurantimonadaceae*, *5 Rhodobiaceae*, *6 Phyllobacteriaceae*, *7 Rhizobiaceae*, *8 Xanthobacteraceae*, *9*, *Bradyrhizobiaceae*, *10 Methylobacteriaceae*, *11 Beijerinckiaceae*, *12 Methylocystaceae*, and *13 Hyphomicrobiaceae*. Table from Hwang and Cho (2008) updated, with permission

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Nitrate reduction	V	V	–	–	V	V	+	V	V	V	NA	+	V
Gelatin hydrolysis	V	–	–	–	–	V	–	V	–	V	NA	V	V
Arginine dihydrolase	V	–	+	+	NA	–	–	V	–	NA	NA	NA	–
Urease	V	V	–	+	NA	V	+	V	+	V	V	V	+
Major quinone[a]	Q-10	Q-10	Q-10	Q-10, Q-9	Q-10, MK-10	Q-10, Q-11	Q-10	Q-10,	Q-10, MK-10, RQ-10	Q-10	Q-10	Q-10, Q-8	Q-10, Q-9, MK-9, RQ-10
Polar lipids ϕ	PC, PG, PE, DPG, PME, GL, ALs, Ls	PC, PG, PE, DPG PME, AL (PS, PN, PLs)	NA	PC, PG, PE, DPG, PME, PDE, Ls	NA	PC, PG, PE, DPG (PME, PDE, ALs, PL)	PC, PG, PE, DPG, PME, PDE	PE, PDE (PC, PG, PA)	PC, PG, PE, DPG, ALs	PC, PG, PE, DPG	PME (PG, PE)	PC, PG, PE, PDE (PME, PS, PL)	PC, PG, PE, DPG, PDE (PA, BPG)
DNA G+C content (mol%)	52–64 %	54.5–59	37–41	57.6–66.3	65.2–65.7	53.1–65.1	57–67.4	65–70	59–69	63.5–72.4	54.7–63.1	61–70	59–71.4

+ Positive, – negative, *V* variable, *NA* data not available

[a]Q Ubiquinone, MK Menaquinone, RQ Rhodoquinone

ϕ Polar lipids in parentheses were observed in <50 % of genera for which data are available in each family. *PC* Phosphatidylcholine, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *BPG* bisphosphatidylglycerol, *PE* phosphatidylethanolamine, *PME* phosphatidylmonomethylethanolamine, *PDE* phosphatidyldimethylethanolamine, *PA* phosphatidic acid, *PS* phosphatidylserine, *PN* aminophospholipid, *PL* unidentified phospholipid, *GL* unidentified glycolipid, *AL* an unidentified aminolipid, *L* an unidentified lipid

The cells are approximately 0.2–0.4 μm wide and 1.0–3.0 μm long. They are weakly motile by a polar flagellum. Reproduction occurs by bussing, binary fission, or asymmetric division. Rosette formation occurs. No growth occurs on trypticase soy agar (TSA), fivefold-diluted TSA, Czapek–Dox agar, MacConkey agar, blood agar, or the above media supplemented with either 3 % (w/v) NaCl or 3 % (w/v) sea salts. Circular, entire, convex and creamy white colonies appear on marine agar 2216 or R2A agar supplemented with 3 % (w/v) NaCl. At optimal growth conditions, colonies are approximately 2 mm in diameter after incubation for 1 week. Intracellular granules of poly-β-hydroxybutyrate are formed. Growth occurs on acetate, α-ketobutyric acid, citrate, D-fructose, D-glucose, mannitol, D-mannose, ribose, sorbitol, glutamic acid, glycerol, glycogen, inositol, inulin, L-arginine, L-asparagine, L-lysine, L-ornithine, *N*-acetylglucosamine, polyethylene glycol, L-pyruvate, sodium succinate, sucrose, thiamine, casamino acids, L-proline, peptone, tryptone and yeast extract. No growth occurs on acetamide, benzoate, DL-cysteine, cellobiose, D-galactose, raffinose, salicin, trehalose, L-xylose, ethanol, formic acid, glycine, 2-propanol, D-lactose, L-arabinose, ascorbate, L-rhamnose, maleic acid, oxalic acid, salicylate, tartrate or urea.

Growth occurs in temperatures between 15 °C and 31 °C (optimum, 25–30 °C), at pH 6–9 (optimum pH 8) and NaCl concentrations of 2–5 % (w/v, optimum 3 %). Decomposition of casein, cellulose, xanthine, hypoxanthine, and hydrolyses of gelatin and aesculin occur. Negative results are observed for nitrate reduction, indole production, arginine dihydrolase and urease. Alkaline phosphatase and trypsin are present. Esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-phosphohydrolase, and *N*-acetyl-β-glucosaminidase activities are weakly present. Lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α- and β-glucosidase, α- and β-galactosidase, α-mannosidase and α-fucosidase activities are absent.

Acid production from glycerol, DL-arabinose, D-ribose, DL-xylose, D-glucose, D-fructose, D-mannose, inositol, D-mannitol, D-sorbitol, *N*-acetylglucosamine, D-lyxose, L-fucose, potassium gluconate, and potassium 5-ketogluconate were observed. Acid production was not observed from erythritol, D-adonitol, methyl β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, D-cellobiose, maltose, D-lactose, melibiose, sucrose, trehalose, inulin,

melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-tagatose, D-fucose, DL-arabitol, or potassium 2-ketogluconate. The cells are sensitive to (μg per disc) gentamicin (6), cephalexin (20), vancomycin (20), mitomycin C (0.6), kanamycin (20), penicillin (6), erythromycin (10), chloramphenicol (20), ciprofloxacin (3), and ampicillin (6). Cells are resistant to tetracycline (20 μg per disc), nalidixic acid (20 μg per disc) and streptomycin (6 μg per disc).

The major fatty acids are $C_{18:1}\omega7c$ (54.3–55.1 %) and $C_{15:0}$ iso 2-OH and/or $C_{16:1}\omega7c$ (summed feature 3; 19.2–20.4 %), $C_{20:1}\omega7c$ (9.6–11.1 %), $C_{18:0}$ (3.1–3.3 %), $C_{14:0}$ 3-OH and/or $C_{16:1}$ iso I (3.0 %), $C_{18:0}$ 3-OH (1.9–2.0 %), $C_{17:1}\omega8c$ (1.1–1.5 %) and $C_{16:0}$ (1.1 %). Other minor fatty acids (<1 %) are $C_{9:0}$, $C_{14:0}$, $C_{17:0}$, $C_{19:0}$, $C_{20:0}$, $C_{14:1}\omega5c$, $C_{19:0}$ cyclo ω8*c*, $C_{16:0}$ 3-OH, $C_{17:0}$ iso 3-OH and $C_{19:0}$ 10-methyl. Besides the polar lipids of the genus, *C. gelatinilyticus* has also minor amounts of an unidentified aminolipid (AL2) and unidentified lipids (L1–4). The type strain, CL-GR15^T (= KCCM 42319^T = DSM 18289^T), was isolated from coastal waters of the east coast of Korea.

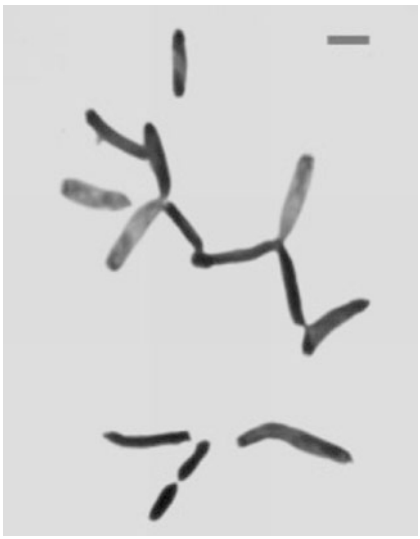

Fig. 8.2
Transmission electron micrograph of negatively stained cells of strain CL-GR15T. Bar, 1 μm (From Hwang and Cho (2008), with permission)

Description of *Cohaesibacter marisflavi*, Qu et al. (2011)

Cohaesibacter marisflavi (ma.ris.fla'vi. L. neut. n. *mare –is* the sea; L. adj. *flavus -a -um* yellow; N.L. gen. n. *marisflavi* of the Yellow Sea, referring to the isolation of the type strain).

The cells are Gram-negative, catalase-negative, oxidase-positive, facultative anaerobic rods that are 0.6–0.7 mm wide and 1.8–2.0 mm long (Fig. 8.2). Cells are motile by a single polar flagella. They reproduce by binary fission or asymmetrical division. Colonies, which grow on 2216E agar medium, are white, smooth, circular, lightly transparent, and 1.0–2.0 μm in diameter after 3–5 days of cultivation at 30 °C. Intracellular poly-b-hydroxybutyrate granules are accumulated. Positive results were obtained in API 20NE tests for indole production, utilization of D-glucose, D-mannose, malic acid, *N*-acetylglucosamine, and trisodium citrate and weakly positive for utilization of β-galactosidase, maltose and L-arabinose; results were negative for gelatin hydrolysis, D-glucose fermentation, arginine dihydrolase, and utilization of D-mannitol, potassium gluconate, capric acid, adipic acid, and phenylacetic acid. In the Biolog GN2 system, the tests were positive for utilization of dextrin, D-fructose, raffinose, glycerol, L-arabinose, *N*-acetyl-D-glucosamine and sucrose. Growth occurs in temperatures between 10 °C and 38 °C (optimum 25–30 °C) at pH 4–9 (optimum pH 7–8) and in 0.5–15 % (w/v) NaCl (optimum 3 %).

In the API ZYM test strip, the results were positive for esterase (C4), naphthol-AS-BIphosphohydrolase, and trypsin; weakly positive for acid phosphatase, alkaline phosphatase, esterase lipase (C8), and leucine aminopeptidase; and negative for cystine aminopeptidase, lipase (C14), *N*-acetyl-β-glucosaminidase, valine aminopeptidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase, and β-glucuronidase. They also have urease and β-glucosidase (aesculin hydrolysis) activities and nitrate reduction. Cells are sensitive to (μg per disc, unless noted) ampicillin (10), carbenicillin (100), cefalexin (30), chloramphenicol (30), ciprofloxacin (5), doxycycline (30), erythromycin (15), kanamycin (30), nalidixic acid (30), neomycin (30), novobiocin (30), penicillin G (10 IU), rifampicin (5), streptomycin (10), and tetracycline (30). Cells are resistant to (μg per disc, unless noted) clindamycin (2), lincomycin (2), oxacillin (1), polymyxin B (300 IU), and vancomycin (30).

The major fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$, $C_{16:0}$ and $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH. Ubiquinone 10 is the major quinone. The DNA G+C content is 55.2 mol%. The type strain, DQHS21^T (=CGMCC 1.9157^T = NCCB 100300^T) was isolated from sediment sampled from a seawater pond used for sea cucumber culture at Jimo, Qingdao province, China, on the west coast of the Yellow Sea.

Genus *Breoghania*, Gallego et al. (2010)

Breoghania (Bre.o.gha'ni.a. N.L. fem. n. *Breoghania*, named after *Breoghan*, according to Celtic mythology (*Leabhar Ghabhala*, XII century), the first Celtic king of *Gallaecia* (actual Galicia), founder of the city of *Brigantia* (probably A. Coruña) who built a tower on the coast from where Eire (Ireland) could be seen.

The members of this genus are Gram-negative, aerobic rods that are motile by polar flagella. Cellular division occurs by binary fission or asymmetric division. They present oxidase and catalase activity. The DNA G+C content of the type strain is 63.9 mol%. The respiratory quinone is ubiquinone 10 (Q-10). The predominant fatty acid is $C_{18:1}\omega7c$ (75.3 %); and other fatty acids found in smaller amounts are $C_{19:0}$ cycloω8c and $C_{16:0}$. The major polar

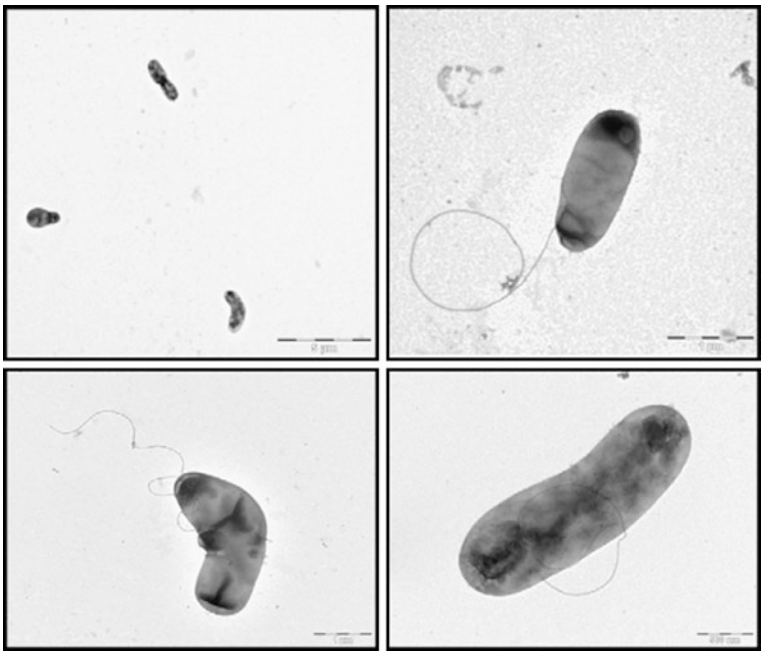

Fig. 8.3
Transmission electron micrographs of negatively stained cells of strain UBFP1T (From Gallego et al. (2010), with permission)

lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, and phosphatidylcholine. The type species is *Breoghania corrubedonensis*.

Description of *Breoghania corrubedonensis*, Gallego et al. (2010)

Breoghania corrubedonensis (Co.ru.be.do.nen'sis N.L. fem. adj. corrubedonensis, of or belonging to Corrubedo, northwest Spain, isolated from the beach of Corrubedo, the location of the sand sample that was used to inoculate the enrichment cultures from which strain UBF-P1^T was isolated).

Cells are regular, irregular, or bulbous rods that are approximately 0.6–0.7 μm wide and 2–3.5 μm long (*Fig. 8.3*). Cells are motile by one or two subpolar flagella. Reproduction occurs by binary fission or asymmetric division. Circular, entire, convex, mucoid, and creamy white colonies appear on LB ASW, LB 3 % NaCl, or marine agar. After incubation for 5 days at optimal growth conditions, colonies are approximately 1 mm in diameter. Growth occurs on glucose, arabinose, mannitol, D-sorbitol, adipate, gluconate (w), phenyl acetate, acetate, succinate, malate, pyruvate, Casamino acids (Difco), acetone, and Tween 20. No growth occurs on D-xylose, mannose, maltose, starch, caprate, *N*-acetyl-glucosamine, citrate, lactate, oxalacetate, propionate, or methanol. Hydrolysis of gelatine or aesculin does not occur. Nitrate is reduced to nitrite, but nitrite reduction does not occur, nor does indole production or H2S formation. β-Glucosidase and β-galactosidase are not produced. Starch, DNA, and Tween 80 are not degraded. Growth occurs in temperatures between 15 °C and 40 °C (optimum 30 °C), at pH 5–8.5 (optimum 7.5) and with NaCl concentrations of 1–10 %. Aminopeptidase, arginine dihydrolase, ornithine decarboxylase, urease, catalase, oxidase, and nitrate reduction are observed. Acid production was weak produced from L-arabinose.

The major fatty acids are $C_{18:1}\omega7c$ (75.3 %), $C_{19:0}$ cyclo $\omega8c$ (6.4 %), $C_{16:0}$ (4.4 %), $C_{16:1}$ $\omega7c$ and/or $C_{15:0}$ iso 2-OH (summed feature 3, 2.5 %), $C_{18:0}$ 3-OH (2.4 %), $C_{18:0}$ (2.2 %), and $C_{20:1}$ $\omega9c$ (1.3 %). Other minor (<1 %) fatty acids are $C_{14:0}$ 3-OH or $C_{16:1}$ iso I (summed feature 2), $C_{17:1}$ $\omega8c$, $C_{17:1}$ $\omega6c$, and $C_{17:0}$. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), and phosphatidylcholine (PC). The DNA G+C content is 63.9 mol%. The type strain was isolated from the beaches of Corrubedo in northwest Spain. It has been deposited in three culture collections under the numbers CECT 7622, LMG 25482, and DSM 23382.

Remarks

The whole genome sequencing of the type strain *Cohaesibacter gelatinilyticus* CL-GR15 is in progress. The South Korean project is sequencing the genome using Illumina Hiseq 2000, 454-GS-FLX (http://www.genomesonline.org/cgi-bin/GOLD/GOLDCards.cgi?goldstamp=Gi0034926).

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