

Cohaesibacter gelatinilyticus gen. nov., sp. nov., a marine bacterium that forms a distinct branch in the order *Rhizobiales*, and proposal of *Cohaesibacteraceae* fam. nov.

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Two novel Gram-negative bacterial strains, designated CL-GR15^T and CL-GR35, were isolated from coastal seawater of the east coast of Korea. Identical 16S rRNA gene sequences were found in the two strains, and it was found that the strains represented a distinct and deep evolutionary lineage of descent in the order *Rhizobiales*, and clustered with yet-uncultured marine bacteria. This lineage could not be associated with any of 12 known families in the order *Rhizobiales*. The most closely related established genus was *Ochrobactrum* (90.7–92.5 % sequence similarity) in the family *Brucellaceae*. Cells of the strains were facultatively anaerobic, weakly motile rods which occasionally exhibited cohesion with each other. The strains were catalase-, oxidase- and gelatinase-positive, and accumulated intracellular poly-β-hydroxybutyrate granules. Ubiquinone 10 was the major quinone. The major polar lipids comprised phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, an unidentified aminolipid and an unidentified glycolipid. The cellular fatty acids mainly consisted of C₁₈:1ω7c, summed feature 3 (C₁₅:0 iso 2-OH and/or C₁₆:1ω7c) and C₂₀:1ω7c. The DNA G+C content was 52.8–53.0 mol%. DNA–DNA hybridization experiments revealed high values (>97 %) for relatedness between strains CL-GR15^T and CL-GR35 and suggested that these two strains constituted a single species. The distinct phylogenetic position and combinations of genotypic, phenotypic and chemotaxonomic characteristics support the proposal of *Cohaesibacter* gen. nov., with the type species *Cohaesibacter gelatinilyticus* sp. nov. (type strain, CL-GR15^T=KCCM 42319^T=DSM 18289^T). *Cohaesibacteraceae* fam. nov. is also proposed.

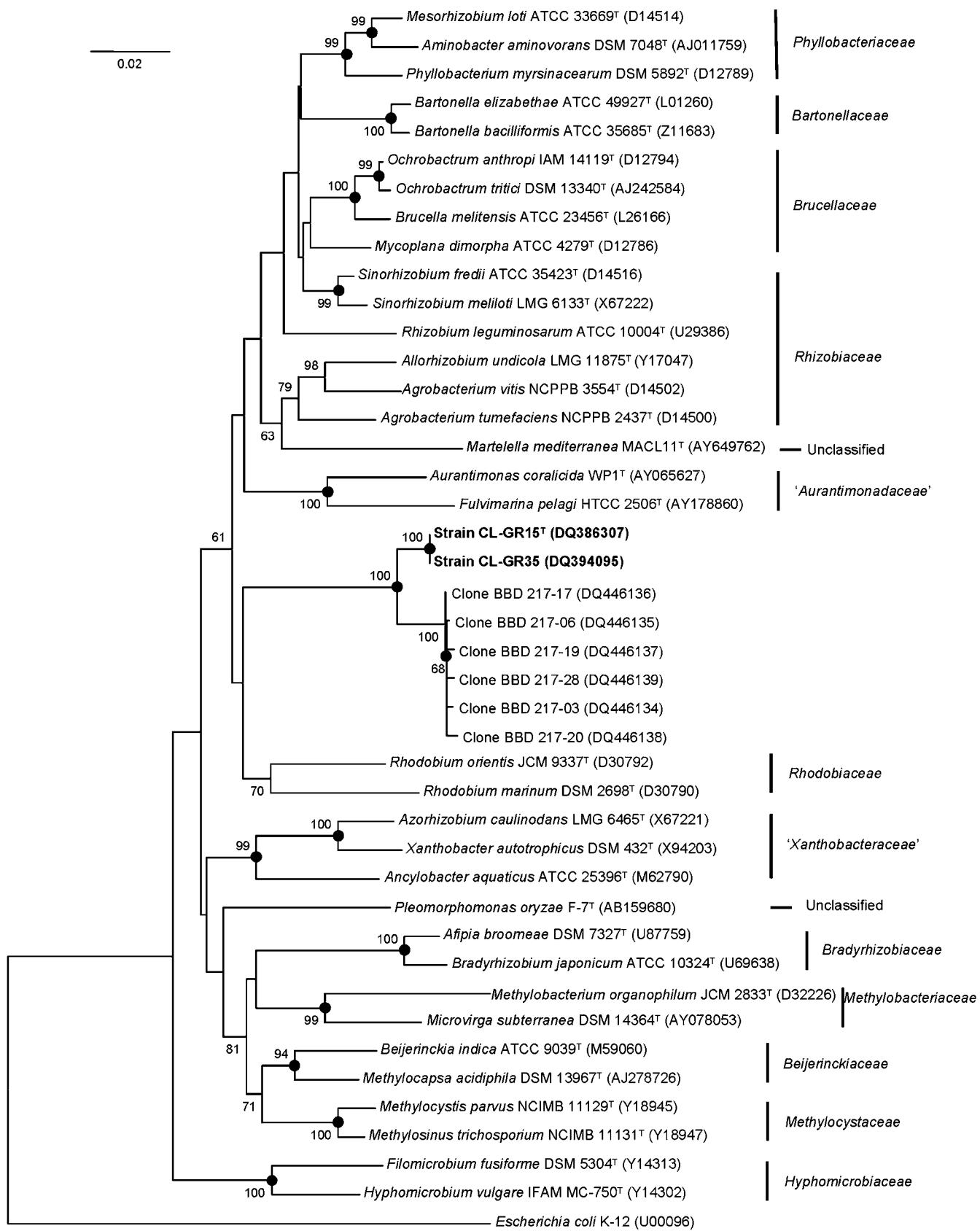
The class *Alphaproteobacteria* comprises the seven orders *Caulobacterales*, ‘*Parvularculales*’, *Rhizobiales*, *Rhodobacterales*, *Rhodospirillales*, *Rickettsiales* and *Sphingomonadales* (Garrity *et al.*, 2005). The order *Rhizobiales* is a phenotypically heterogeneous assemblage within the α-2 subgroup of the *Proteobacteria* (Woese *et al.*, 1984), and is divided into 10 families on the basis of 16S rRNA gene sequence analyses (Kuykendall, 2005a): *Rhizobiaceae*, *Bartonellaceae*, *Brucellaceae*, *Phyllobacteriaceae*, *Methylocystaceae*, *Beijerinckiaceae*, *Bradyrhizobiaceae*, *Hypomicrobiaceae*, *Methylobacteriaceae* and *Rhodobiaceae*. In addition to these families, a new family was described as ‘*Aurantimonadaceae*’ and contains the genera *Aurantimonas* and *Fulvimonas* within the order *Rhizobiales*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-GR15^T (=KCCM 42319^T=DSM 18289^T) is DQ386307.

Supplementary figures are available with the online version of this paper.

(Garrity *et al.*, 2004). Recently, phylogenetic analysis of 16S rRNA gene sequences excluding six variable regions for 261 species in the class *Alphaproteobacteria* revealed another family, ‘*Xanthobacteraceae*’, within the order *Rhizobiales* (Lee *et al.*, 2005). In this paper, two strains representing a novel family-level lineage within the order *Rhizobiales* were isolated and characterized by polyphasic approaches (Vandamme *et al.*, 1996).

A coastal water sample was incubated with sand sediment in a 150 mm diameter glass Petri dish at room temperature for about 5 months. Without disturbing the sediment, 100 µl superficial seawater was taken and spread on a plate containing R2A agar (Difco) supplemented with 3 % (w/v) NaCl, and the plate was incubated at 25 °C for 5 days. Strains CL-GR15^T and CL-GR35 were isolated from the same plate and subsequently purified on R2A agar supplemented with 3 % (w/v) NaCl at 25 °C four times. The strains were able to grow well on marine agar 2216 (MA; Difco) as well as R2A agar supplemented with 3 %



(w/v) NaCl. The strains were maintained on MA at 25–30 °C and stored frozen at –80 °C as described by Choi *et al.* (2006).

The 16S rRNA gene was amplified from a single colony by using *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using the *AccuPrep* PCR Purification kit (Bioneer), and direct sequence determination of the purified 16S rRNA gene was performed with an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen (Seoul, Korea). The almost-complete 16S rRNA gene sequences of strains CL-GR15^T (1374 bp) and CL-GR35 (1344 bp) were obtained and compared with those available in GenBank using BLASTN searches (Altschul *et al.*, 1990). The sequences of strains CL-GR15^T and CL-GR35 were manually aligned with those of 189 species with validly published names belonging to related families in the order *Rhizobiales*, obtained from GenBank and the Ribosomal Database Project (Cole *et al.*, 2003) databases, using known 16S rRNA secondary structure information. Phylogenetic trees were obtained by neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of tree topologies was assessed by bootstrap analyses based on 1000 replications for neighbour-joining and maximum-parsimony methods. Alignment analysis was carried out using the jPHYDIT program (Jeon *et al.*, 2005), and phylogenetic analyses were carried out using MEGA3 (Kumar *et al.*, 2004).

Signature nucleotides present within 16S rRNA genes were searched for using the sequences aligned for the phylogenetic analysis. Only those positions were selected for signatures for which the majority of families each exhibited a single nucleotide. Every signature nucleotide found was then positioned on the secondary structure of the 16S rRNA molecule of *Escherichia coli* (Ivanova *et al.*, 2004).

Morphological and physiological tests were performed as follows. Gram-staining was performed as described by Smibert & Krieg (1994). Cellular morphology and the presence of flagellum were observed using transmission electron microscopy (EX2; JEOL). Motility was determined by the hanging drop method (Smibert & Krieg, 1994). Anaerobic growth was checked on MA using the GasPak anaerobic system (BBL). Bacteriochlorophyll *a* production was determined in acetone extracts from cells cultured in the light and the dark, and examined with an Ultraspec 2000 spectrophotometer (Pharmacia Biotech). Poly-β-hydroxybutyrate granules were observed by epifluorescence microscopy (BX60; Olympus) after Nile blue A staining

(Ostle & Holt, 1982). Growth was tested on trypticase soy agar (TSA; BBL), fivefold-diluted TSA, Czapek–Dox agar (Weyland, 1969), MacConkey agar (Difco) and blood agar (Difco) or the above media supplemented with either 3% (w/v) NaCl or 3% (w/v) sea salts (Sigma). The temperature range for growth was tested on the basis of colony formation on MA plates incubated at 5, 10, 15, 20, 25, 30, 31, 33, 35 and 40 °C. The pH range (pH 4–10 at intervals of 1 pH unit) for growth was determined both on the basis of colony formation on MA plates and assessing changes in OD₆₀₀ in pH-buffered marine broth medium (Difco) (Manaia *et al.*, 2003). To test salt tolerance, R2A agar (Difco) containing various concentrations of NaCl [0, 0.5, 1, 2, 3, 5, 7, 10, 15, 20, 25 and 30% (w/v)] were used. Catalase and oxidase activities and degradation of casein, cellulose, xanthine and hypoxanthine were determined according to the protocols described by Smibert & Krieg (1994). In addition, nitrate reduction, the production of indole, arginine dihydrolase, urease, gelatinase and the hydrolysis of aesculin were tested using the API 20NE kit (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was supplemented with NaCl (3%, w/v). Other enzyme activities and acid productions were assayed using the API ZYM and the API 50CH kits (bioMérieux), respectively, with 3% NaCl solution as the suspension medium. Carbon utilization was tested using basal medium supplemented with 0.01% (w/v) yeast extract (Tris/HCl, 50 mM, pH 7.5; NH₄Cl, 19 mM; K₂HPO₄.3H₂O, 0.33 mM and FeSO₄.7H₂O, 0.1 mM in artificial seawater; Baumann & Baumann, 1981) containing 0.1% carbon source based on changes in OD₆₀₀ over 4 weeks. Sensitivities to antibiotics were carried out using disc-diffusion methodology (Bauer *et al.*, 1966). All phenotypic assays (except analysis of the temperature range) were carried out at 25–30 °C, which was the range of optimal growth temperature for strains CL-GR15^T and CL-GR35.

Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). The fatty acid methyl esters in whole cells, which were grown on MA at 30 °C for 4 days, were analysed by gas chromatography according to the instruction of the Microbial Identification System (MIDI) at the Korean Culture Center of Microorganisms (KCCM) in Seoul, Korea. The quinone system was determined according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985). The DNA G+C content was analysed by HPLC (HP 100; Hewlett Packard) analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), after DNA extraction

Fig. 1. Neighbour-joining tree showing relationships between the strains studied and representatives of related families in the order *Rhizobiales*. Only bootstrap values (expressed as percentage of 1000 replications) greater than 60% are shown at nodes. Solid circles indicate that the corresponding nodes are also recovered in maximum-parsimony trees. Bar, 0.02 nucleotide substitutions per site.

Table 1. Selected 16S rRNA gene signature nucleotides for the families of the order *Rhizobiales*

Lower-case letters indicate bases found in less than 50 % of assayable cases. Positions follow *E. coli* numbering.

Position	129–232	194	297	600–638	678–712	1244–1293
<i>Cohaesibacteraceae</i> fam. nov.	A–G	T	G	A–T	A–T	A–T
<i>Brucellaceae</i>	A–G	G	G	A–T	T–A	G–C
<i>Bartonellaceae</i>	A–G	G	G	A–T	T–A	G–C
‘ <i>Aurantimonadaceae</i> ’	A–G	G	A	A–T	T–A	G–C
<i>Rhodobiaceae</i>	A–G	G	G	A–T	T–A	G–C
<i>Phyllobacteriaceae</i>	A–G	G	G	A–T	T–A	G–C
<i>Rhizobiaceae</i>	A–G	G	G	A–T	T–A	G–C
‘ <i>Xanthobacteraceae</i> ’	G–A	G	G	A–T	T–A	G–C
<i>Bradyrhizobiaceae</i>	A–G	G	G	A(g)–T(c)	T–A	G–C
<i>Methylobacteriaceae</i>	A–G	G	G	G–C	T–A	G–C
<i>Beijerinckiaceae</i>	A–G	G	G	A–T	T–A	G–C
<i>Methylocystaceae</i>	A(g)–G(a)	G	G	A–T	T–A	G–C
<i>Hyphomicrobiaceae</i>	A(g)–G(a)	G	G	A(g)–T(c)	T–A	G(a)–C(t)

by the method of Marmur (1961). Lambda DNA was used as a standard. The relatedness of genomic DNA between strains CL-GR15^T and CL-GR35 was determined by dot-blot hybridization as described by Choi *et al.* (2006).

Direct sequencing of the 16S rRNA gene revealed that sequences of strains CL-GR15^T and CL-GR35 were identical, and showed 96.2–96.4 % sequence similarity to clones obtained from black band-diseased corals located at water depths of 24 and 27 m (Sekar *et al.*, 2006; Fig. 1). Sequence comparisons to representative bacteria with validly published names indicated that strains CL-GR15^T and CL-GR35 belonged to the order *Rhizobiales* in the *Alphaproteobacteria* (Fig. 1; see a global tree available as Supplementary Fig. S1 in IJSEM Online). The strains were most closely related to the genera *Ochrobactrum* and *Brucella* in the family *Brucellaceae* (90.9–92.5 % similarity), the genus *Sinorhizobium* in the family *Rhizobiaceae* (90.9–91.5 %), the genus *Bartonella* in the family *Bartonellaceae* (90.6–91.9 %), the genus *Rhodobium* in the family *Rhodobiaceae* (89.3–91.2 %) and the genus *Phyllobacterium* in the family *Phyllobacteriaceae* (89.3–91.2 %). In all phylogenetic trees, strains CL-GR15^T and CL-GR35, together with the recently observed clone sequences from black band-diseased corals (Sekar *et al.*, 2006), form a distinct monophyletic clade with 100 % bootstrap within the order *Rhizobiales* (Fig. 1, Supplementary Fig. S1). Further, this lineage could not be associated with any of the 12 known families and two unclassified genera in the order *Rhizobiales*. In addition, ten 16S rRNA gene signature nucleotides for the families of the order *Rhizobiales* were detected (Table 1). Strains CL-GR15^T and CL-GR35 contained three signature nucleotides that are types of compensatory transversion mutations (positions 678–712; Table 1) and a single transversion mutation (position 194; Table 1). Therefore, strains CL-GR15^T and CL-GR35 could be recognized as representing a distinct genus belonging to a new family within the order *Rhizobiales*.

Strains CL-GR15^T and CL-GR35 gave identical results for morphological, physiological and biochemical characteristics, and the results are given in the genus and species descriptions, Fig. 2, Supplementary Fig. S2 and Table 2. The DNA G+C content was 52.8–53.0 mol%. DNA–DNA hybridization experiments revealed high values (>97 %) for relatedness between strains CL-GR15^T and CL-GR35. DNA–DNA hybridization, phenotypic and chemotaxonomic characteristics revealed that they belonged to the same species.

In addition to phylogenetic differentiation of strains CL-GR15^T and CL-GR35 from the other families of the order *Rhizobiales*, combinations of phenotypic and chemotaxonomic characteristics can differentiate the strains from members of other families (Table 2). The difference of G+C contents between these strains and other species belonging to the families *Bartonellaceae*, *Rhodobiaceae*, ‘*Xanthobacteraceae*’ and *Methylobacteriaceae* was >10 mol% (Table 2), showing a distinct genetic difference. The major quinone for strains CL-GR15^T and CL-GR35

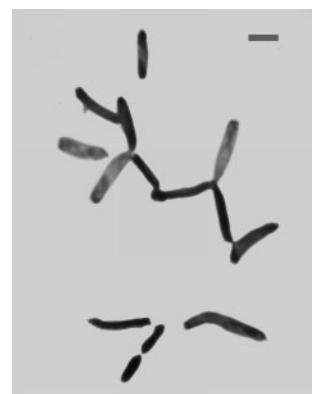


Fig. 2. Transmission electron micrograph of negatively stained cells of strain CL-GR15^T. Bar, 1 µm.

Table 2. Differential characteristics of the families in the order *Rhizobiales*

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*. Data are obtained from: 1, this study; 2, Dees *et al.* (1981), Coloe *et al.* (1984), Ewalt *et al.* (1994), Kämpfer *et al.* (2003), Corbel & Banai (2005), Holmes (2005), Trujillo *et al.* (2005), Urakami & Segers (2005), Kämpfer *et al.* (2006), Tripathi *et al.* (2006) and Teyssier *et al.* (2007); 3, Welch *et al.* (1992), Bermond *et al.* (2000, 2002), Dehio *et al.* (2001) and Welch (2005); 4, Cho & Giovannoni (2003), Denner *et al.* (2003) and Jurado *et al.* (2006); 5, Imhoff & Thiemann (1991), Hiraishi *et al.* (1995) and Imhoff & Hiraishi (2005); 6, Bambauer *et al.* (1998), Kämpfer *et al.* (2002), Mergaert *et al.* (2002), Gao *et al.* (2004), Labbé *et al.* (2004), Schleheck *et al.* (2004), Chen *et al.* (2005), Jurado *et al.* (2005), Kämpfer (2005), Mergaert & Swings (2005), Peix *et al.* (2005), Staley *et al.* (2005a, b), Urakami (2005), Valverde *et al.* (2005) and Mantelin *et al.* (2006); 7, Thompson *et al.* (1983), Tighe *et al.* (2000), Wang *et al.* (2002), Im *et al.* (2004), Balkwill (2005), Kuykendall *et al.* (2005) and Young *et al.* (2005); 8, Kelly *et al.* (2000), Xin *et al.* (2004), Kuykendall (2005c), Miller *et al.* (2005), Vasilyeva (2005b) and Wiegel (2005); 9, Hougaard *et al.* (2000), La Scola *et al.* (2002, 2003), Zhang *et al.* (2002), Das (2005), Imhoff (2005a, b), Kennedy (2005b), Kuykendall (2005b), Meyer (2005), Sly & Hugenholtz (2005), Speck & Bock (2005) and Weyant & Whitney (2005); 10, Rihs *et al.* (1993), Kanso & Patel (2003), Green (2005), Jiang *et al.* (2006) and Aslam *et al.* (2007); 11, Dedysh *et al.* (2000, 2002), Dunfield *et al.* (2003), Egli & Auling (2005), Kennedy (2005a) and Staley *et al.* (2005c); 12, Doronina *et al.* (1998, 2001), Doronina & Trotsenko (2005a) and Bowman (2005a, b); 13, Rivas *et al.* (2003), Doronina & Trotsenko (2005b), Gliesche *et al.* (2005), Hiraishi & Imhoff (2005), Hirsch & Gebers (2005), Imhoff (2005c, d), Jenkins *et al.* (2005a, b), Miller *et al.* (2005), Nakagawa *et al.* (2005), Schlesner (2005), Vasilyeva (2005a), Yoo *et al.* (2006) and Yoon *et al.* (2007). +, Positive; –, negative; v, variable; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Nitrate reduction	–	v	–	–	v	v	+	v	v	v	NA	+	v
Gelatin hydrolysis	+	–	–	–	–	v	–	v	–	v	NA	v	v
Arginine dihydrolase	–	–	+	+	NA	–	–	v	–	NA	NA	NA	–
Urease	–	v	–	+	NA	v	+	v	+	v	v	v	+
Major quinone*	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,	Q-10	Q-10, Q-8	Q-10, Q-9, MK-9, RQ-10	RQ-10
Polar lipids†	PC, PG, PE, PC, PG, PE, DPG, PME, DPG PME, GL, ALs, Ls AL (PS, PN, PLs)	NA	PC, PG, PE, DPG, PME, PDE, Ls	NA	PC, PG, PE, PC, PG, PE, DPG (PME, DPG, PME, PDE, ALs, PDE, PA, PL)	PE, PDE (PC, PG, DPG, ALs, PDE, PA)	PC, PG, PE, PC, PG, PE, DPG (PE)	PC, PG, PE, PC, PG, PE, DPG (PE)	PME (PG, PE)	PC, PG, PE, DPG, PE, PA)	PDE (PME, PS, PL)	PC, PG, PE, DPG, PDE (PA, BPG)	
DNA G+C content (mol%)	52.8–53.0	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

*Q, Ubiquinone; MK, menaquinone; RQ, rhodoquinone.

†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, phosphatidylmonomethyl ethanolamine; PDE, phosphatidyldimethyl ethanolamine; PA, phosphatidic acid; PS, phosphatidylserine; PN, aminophospholipid; PL, unidentified phospholipid; GL, unidentified glycolipid; AL, an unidentified aminolipid; L, an unidentified lipid.

was ubiquinone-10 (Q-10), which is typically observed in the *Alphaproteobacteria* as the major quinone (Collins & Jones, 1981; Kämpfer *et al.*, 2003). However, the sole major respiratory quinone (Q-10) can differentiate strains CL-GR15^T and CL-GR35 from the families '*Aurantimonadaceae*', *Phyllobacteriaceae*, *Rhodobiaceae*, *Bradyrhizobiaceae*, *Methylocystaceae* and *Hyphomicrobiaceae* (Table 2). Polar lipid compositions conservatively differentiate the strains from the other 11 families in the order *Rhizobiales*, except the families *Brucellaceae* and *Phyllobacteriaceae* (Table 2). In particular, strains CL-GR15^T and CL-GR35 can be differentiated from species in the family *Brucellaceae*, which is the most closely related family to the two strains, by the presence of gelatinase and the inability to grow on blood agar. An unidentified aminolipid (AL1) in the profile of strain CL-GR15^T (Supplementary Fig. S3) apparently exhibited the same position as that of AL1, AL and AL1 in the polar lipid profiles of *Ochrobactrum gallinifacetus*, *Ochrobactrum pseudintermedium* and *Pseudochrobactrum asaccharolyticum*, respectively, belonging to the family *Brucellaceae* (Kämpfer *et al.*, 2003, 2006; Teyssier *et al.*, 2007). However, the presence of a significant amount of an unidentified glycolipid could clearly distinguish strain CL-GR15^T from the above species in the family *Brucellaceae*. Overall, strains CL-GR15^T and CL-GR35 cannot be assigned to any previously recognized bacterial family or genus and thus can be described as a novel species within a new genus, *Cohaesibacter gelatinilyticus* gen. nov., sp. nov., in a new family, *Cohaesibacteraceae* fam. nov.

Description of *Cohaesibacteraceae* fam. nov.

Cohaesibacteraceae (Co.hae.si.bact.er.a'ceae. 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).

On the basis of 16S rRNA gene sequence analysis and combinations of genotypic, phenotypic and chemotaxonomic characteristics, the family is a member of the order *Rhizobiales* in the class *Alphaproteobacteria* with the following nucleotide sequence characteristics: 194 (T), 678 (A) and 712 (T). The type genus is *Cohaesibacter*.

Description of *Cohaesibacter* gen. nov.

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].

Cells are Gram-negative, facultatively anaerobic rods. Oxidase- and catalase-positive. The predominant fatty acids are C₁₈:1ω7c and C₁₅:0 iso 2-OH and/or C₁₆:1ω7c and C₂₀:1ω7c. The respiratory quinone is Q-10. The DNA G+C content of the type species is approximately 53 mol%. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethyllethanolamine,

an unidentified aminolipid (AL1) and an unidentified glycolipid. The genus is a member of the family *Cohaesibacteraceae*. The type species is *Cohaesibacter gelatinilyticus*.

Description of *Cohaesibacter gelatinilyticus* sp. nov.

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).

In addition to the characteristics reported for the genus, cells are weakly motile by a polar flagellum and approximately 0.2–0.4 µm wide and 1.0–3.0 µm long. Reproduce by budding or binary fission or asymmetric division. Rosette formation occurs. Intracellular granules of poly-β-hydroxybutyrate are formed. No growth occurs on 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. On MA or R2A agar supplemented with 3% (w/v) NaCl, colonies are circular, entire, convex and creamy white in colour. After incubation for 1 week at optimal growth conditions, colonies are approximately 2 mm in diameter. Grows at temperatures in the range 15–31 °C (optimum, 25–30 °C) and at pH 6–9 (optimum pH 8). Growth occurs with NaCl concentrations of 2–5% (w/v, optimum 3%). Casein, cellulose, xanthine and hypoxanthine are decomposed. Hydrolyses gelatin and aesculin. Negative for nitrate reduction, indole production, arginine dihydrolase and urease. Alkaline phosphatase and trypsin activities are present, and esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-phosphohydro-lase 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 is produced 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, but not 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. 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. Cells are sensitive to (μ g 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), but resistant to tetracycline (20), nalidixic acid (20) and streptomycin (6). The 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 $\omega8c$, $C_{16:0}$ 3-OH, $C_{17:0}$ iso 3-OH and $C_{19:0}$ 10-methyl. In addition to the polar lipids listed in the genus description, minor amounts of an unidentified aminolipid (AL2) and unidentified lipids (L1–4) can be detected.

The type strain, CL-GR15^T (=KCCM 42319^T=DSM 18289^T), was isolated from coastal water of the east coast of Korea.

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