Algoriphagus antarcticus sp. nov., a novel psychrophile from microbial mats in Antarctic lakes

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A taxonomic study was performed on six strains isolated from microbial mats of lakes Reid, Fryxell and Ace in Antarctica. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these strains belonged to the family 'Flexibacteraceae' and were closely related to the recently described genera *Algoriphagus* and *Hongiella*. The isolates were Gram-negative, chemoheterotrophic, aerobic, psychrophilic, orange-red-pigmented bacteria and their DNA G+C content ranged from 39.9 to 41.0 mol%. Whole-cell fatty acid profiles included mainly branched fatty acids and summed feature 3, comprising 15:0 iso 2OH, 16:1 ω 7c or both. On the basis of genotypic, phenotypic, chemotaxonomic and phylogenetic results, the novel strains were classified as *Algoriphagus antarcticus* sp. nov. The type strain is LMG 21980^T (=DSM 15986^T=R-10710^T).

Members of the *Cytophaga*–*Flavobacterium* cluster constitute one of the dominant bacterial groups in the marine environment (Bowman *et al.*, 1997; Pinhassi *et al.*, 1997; Glöckner *et al.*, 1999) and it is now thought that they play an important role in remineralization processes in aquatic systems (Kirchman, 2002). Recently, new genera of the 'Flexibacteraceae' have been described such as *Reichenbachia*, *Algoriphagus*, *Hongiella* and *Belliella* (Nedashkovskaya *et al.*, 2003; Bowman *et al.*, 2003; Yi & Chun, 2004; Brettar *et al.*, 2004) isolated from sea water, sea ice, algal mats of saline lakes and tidal flat sediment. Only one strain (strain A230 of *Algoriphagus ratkowskyi*) was isolated from a cyanobacterial mat sample from Ace Lake, Antarctica.

During the MICROMAT project (November 1998–February 2001), 746 bacterial strains were isolated under heterotrophic conditions from microbial mat samples collected from ten Antarctic lakes in the Vestfold Hills (lakes Ace, Druzhby, Grace, Highway, Pendant, Organic and Watts), the Larsemann Hills (Lake Reid) and the McMurdo Dry Valleys (Lakes Hoare and Fryxell) (Van Trappen *et al.*, 2002). Numerical analysis of their fatty acid composition revealed 41 clusters, and 16S rRNA gene sequence analysis, performed on representative strains, showed that they belong to the α -, β - and γ -subclasses of the

Proteobacteria, the high- and low-G+C Gram-positives and to the phylum 'Bacteroidetes' (Van Trappen *et al.*, 2002).

In the present work, we have studied the taxonomic relationship of six strains from fatty acid cluster 15 (as delineated by Van Trappen *et al.*, 2002), using a polyphasic taxonomic approach. A novel species of the genus *Algoriphagus* is described as *Algoriphagus antarcticus* sp. nov.

The investigated strains were LMG 21980^T (=DSM 15986^T=R-10710^T), LMG 21981 (=R-10749), LMG 21982 (=R-10752) and LMG 21983 (=R-11427) from Lake Reid, Larsemann Hills, Antarctica, LMG 21984 (=R-8290) from Lake Fryxell, McMurdo Dry Valleys, Antarctica, and LMG 21482 (=R-9286) from Ace Lake, Vestfold Hills, Antarctica, and were isolated as described by Van Trappen *et al.* (2002). The strains were routinely cultivated on marine agar 2216 (Difco) at 20 °C for 4 days, except when mentioned otherwise. Strains LMG 21435^T (*A. ratkowskyi*), LMG 21969^T (*Algoriphagus winogradskyi*), LMG 21970^T (*Algoriphagus chordae*) and LMG 21971^T (*Algoriphagus aquimarinus*) were routinely cultivated on marine agar 2216 (Difco) at 20 °C, whilst strain LMG 22067^T (*Algoriphagus halophilus*) was cultivated on marine agar 2216 (Difco) at 28 °C.

DNA extracts were prepared using the method of Pitcher *et al.* (1989). The almost-complete 16S rRNA gene sequences (1462–1491 nucleotides) of strains LMG 21482, LMG 21980^T and LMG 21983 were amplified by PCR using conserved primers (Coenye *et al.*, 1999). PCR products were purified using the QIAquick PCR Purification kit (Qiagen) according to the instructions of the manufacturer.

Published online ahead of print on 23 April 2004 as DOI 10.1099/ij.s.0.02973-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 21482, LMG 21980^T and LMG 21983 are AJ441012/AJ577267, AJ577141 and AJ577142, respectively.

Sequence analysis was performed using an automatic DNA sequencer (ABI Prism 3100; Applied Biosystems), applying a BigDye Terminator Cycle Sequencing v2.0 Ready Reaction kit (Applied Biosystems), following the protocols of the manufacturer. Sequence assembly was performed using the program AutoAssembler 1.4.0 (Applied Biosystems). The closest related sequences were found using the FASTA program, the sequences from reference strains were aligned and editing of the alignment and reformatting was performed using the BioEdit program (Hall, 1999) and ForCon (available at http://www.ebi.ac.uk/embnet.news/vol6_1/ForCon/body_forcon.html) (Raes & Van De Peer, 1999). Evolutionary distances were calculated using the Jukes and Cantor evolutionary model and a phylogenetic tree (Fig. 1) was constructed using the neighbour-joining method (Saitou & Nei, 1987) with the TREECON program (Van De Peer & De Wachter, 1994). Dendrograms obtained by maximum-parsimony and maximum-likelihood analyses showed essentially the same topography (data not shown).

The results of the phylogenetic analysis revealed that the novel strains belonged to the recently described genus *Algoriphagus* within the family 'Flexibacteraceae' (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004), which is most closely related to the genera *Hongiella*, *Belliella* and *Cyclobacterium* (see Fig. 1). The Antarctic strains formed

a robust branch, supported by a high bootstrap value (100 % of the bootstrap replications for all methods).

The 16S rRNA gene sequences of strains LMG 21980^T and LMG 21983 were identical to each other, whilst the sequence of LMG 21482 differed by only one base from these sequences (99.9 % similarity). The 16S rRNA gene sequences of the novel strains showed 98.7 % sequence similarity to that of *A. chordae*, 98.7 % to that of *A. ratkowskyi*, 98.6 % to that of *A. winogradskyi*, 98.5 % to that of *A. aquimarinus*, 97.4 % to *A. halophilus*, 94.7 % to that of *Hongiella ornithinivorans*, 93.8 % to that of *Hongiella mannitolivorans*, 92.9 % to that of *Belliella baltica*, 92.9 % to that of *Cyclobacterium marinum* and less than 90 % to sequences of other related genera.

Genomic relatedness between the novel strains and their most closely related phylogenetic neighbours (*A. ratkowskyi*, *A. chordae*, *A. aquimarinus*, *A. winogradskyi* and *A. halophilus*) was determined by DNA–DNA hybridizations, carried out with photobiotin-labelled probes in microplate wells as described by Willems *et al.* (2001), using an HTS7000 Bio Assay Reader (Applied Biosystems) for the fluorescence measurements. The hybridization temperature was 34 °C and reciprocal experiments were performed for every pair of strains. The mean hybridization level

Fig. 1. Neighbour-joining dendrogram showing the estimated phylogenetic relationships of *A. antarcticus* and related members of the family 'Flexibacteraceae' on the basis of 16S rRNA gene sequences. *Bacteroides fragilis* was chosen as the outgroup. Bootstrap values are shown in percentages of 500 replicates when more than 50 %. Bar, 1 nucleotide substitution per 10 nucleotides. The GenBank/EMBL/DBJ accession number for each reference strain is shown in parentheses.

between the strains LMG 21980^T, LMG 21981, LMG 21982, LMG 21983 and LMG 21984 ranged from 89.0 to 98.7 %, while strain LMG 21482 showed a hybridization value of only 74.2 ± 3.9 % with strain LMG 21980^T and 72.7 ± 0.5 % with strain LMG 21983. Hybridization values of LMG 21980^T and LMG 21983 with the nearest phylogenetic neighbours *A. ratkowskyi* (LMG 21435^T), *A. chordae* (LMG 21970^T), *A. aquimarinus* (LMG 21971^T) and *A. winogradskyi* (LMG 21969^T) ranged from 20.5 to 38.6 %. The hybridization value between the strains LMG 21983 and LMG 22067^T (*A. halophilus*) was only 7.2 %. Differences between reciprocal experiments were less than 8 %. These results clearly showed that the Antarctic strains represented a novel species within the genus *Algoriphagus*.

The G + C content of the DNA from the novel strains was determined using an HPLC method, as described by Van Trappen *et al.* (2003). The G + C values of strains LMG 21482, LMG 21980^T, LMG 21981, LMG 21982, LMG 21983 and LMG 21984 were 40.8, 40.6, 40.6, 40.6, 39.9 and 41.0 mol%, respectively. These values are consistent with the G + C content of the genus *Algoriphagus*, which

ranges from 35 to 42 mol% (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004).

Cellular fatty acid patterns of the novel strains were based on the data generated by Van Trappen *et al.* (2002). The strains showed similar fatty acid profiles and the mean composition was 3.4 % 15:0 anteiso, 23.1 % 15:0 iso, 4.1 % 15:0 iso 3OH, 9.3 % 15:1 iso, 1.8 % 16:0 3OH, 1.9 % 16:0 iso, 2.5 % 16:0 iso 3OH, 2.9 % 16:1 iso, 4.9 % 16:1 ω 5c, 12.5 % 17:0 iso 3OH, 3.4 % 17:1 ω 9c iso, 24.3 % 15:0 iso 2OH and/or 16:1 ω 6c. Other fatty acids each accounted for less than 1 %. The fatty acid profiles of the novel strains resembled those determined for the other *Algoriphagus* species but differed in terms of relative amounts of 15:0 iso, 15:1 iso, 17:0 iso 3OH and 15:0 iso 2OH/16:1 ω 6c (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004).

Morphological, physiological and biochemical tests were performed, as described earlier (Van Trappen *et al.*, 2003). The strains showed typical morphological characteristics of the genus *Algoriphagus* (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004) and their physiological and

Table 1. Phenotypic characteristics differentiating *Algoriphagus antarcticus* sp. nov. and related species

Species: 1, *A. antarcticus*; 2, *A. winogradskyi*; 3, *A. chordae*; 4, *A. aquimarinus*; 5, *A. ratkowskyi*; 6, *A. halophilus*; 7, *H. mannitolivorans*; 8, *H. ornithinivorans*; 9, *B. baltica*; 10, *C. marinum*. Symbols: +, positive test; –, negative test; v, variable between strains; ND, no available data; O, orange; R, red; P, pink. Data from Bowman *et al.* (2003), Yi & Chun (2004), Brettar *et al.* (2004), Nedashkovskaya *et al.* (2004) and this study.

	1	2	3	4	5	6	7	8	9	10
Coiled, ring-shaped, spiral cells	–	–	–	–	–	–	–	–	–	+
Colour of cell mass	O–R	O–R	O–R	O–R	P	O–R	O	O	P–O	P–R
Growth on/at:										
10 % NaCl	–	–	+	+	–	+	–	+	–	+
25 °C	+	+	+	+	+	+	+	+	+	+
30 °C	–	+	+	+	–	+	+	+	+	+
Nitrate reduction	–	+	–	–	–	–	+	–	+	–
Production of:										
Oxidase	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	–	+	+	+	+	+
Acid from carbohydrates	–	+	+	+	+	–	–	–	v	+
Hydrolysis of:										
Agar	–	+	+	+	–	–	–	–	ND	–
Aesculin	+	+	+	+	ND	+	+	+	+	ND
Alginate	–	+	+	+	ND	–	–	–	ND	ND
Casein	–	–	–	+	+	–	–	–	–	–
Chitin	–	–	–	–	v	–	–	–	–	–
CM-cellulose	–	–	–	–	–	–	–	–	–	ND
DNA	–	–	–	+	–	–	+	+	+	–
Gelatin	–	+	–	+	–	+	+	+	–	–
Starch	–	+	–	–	v	–	+	+	+	–
Tween 80	ND	–	–	+	–	+	–	+	ND	–
Urea	–	–	–	–	–	–	–	–	ND	–
G + C content (mol%)	40	41	40	41	35	37	42	38	35	36

biochemical characteristics are given in the description. The results of the polyphasic analysis supported the recognition of a novel species within the genus *Algoriphagus* and the name *Algoriphagus antarcticus* sp. nov. is proposed. The novel species can be clearly differentiated from related *Algoriphagus* and *Hongiella* species by several phenotypic characteristics (see Table 1).

Description of *Algoriphagus antarcticus* sp. nov.

Algoriphagus antarcticus (ant.arc'ti.cus. L. masc. adj. *antarcticus* of the Antarctic, the environment from where the strains were isolated).

The rod-shaped cells range from 2 to 3 µm in length and up to 0.5 µm in width; motility not detected. The strains grow at 5–20 °C, with weak growth at 25 °C and an optimal growth temperature of 20 °C. No growth occurs at 30 °C. Orange-red-pigmented, convex, opaque colonies with a diameter of 0.5–3 mm with entire margins are formed on marine agar plates after 6 days incubation. Colonies on Anacker & Ordal's agar are flat, round with entire margins and 0.5–0.7 mm in diameter after 14 days incubation. Growth also occurs on nutrient agar and R2A and colonies do not adhere to the agar. No growth on trypticase soy agar. Gram-negative and catalase- and oxidase-positive. Degrades aesculin. Growth is not observed (API 20NE) on glucose, mannose, maltose, L-arabinose, mannitol, N-acetylglucosamine, gluconate, caprate, adipate, malate, citrate and phenylacetate. Acids are not produced from carbohydrates (API 20E). Agar, alginate, pectin, chitin, casein, CM-cellulose, DNA, starch, gelatin, tyrosine and urea are not degraded. Congo red is not absorbed. There is no production of a brown diffusible pigment on L-tyrosine agar and no precipitate is formed on egg-yolk agar. Tests for indole production, citrate utilization, nitrate reduction, the Voges-Proskauer reaction and hydrogen sulfide production are negative. None of the strains shows activity for the enzymes arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase (API 20E), lipase (C14), cystine arylamidase (except strain LMG 21983), α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase (API ZYM). Weak enzymic activity is observed for esterase (C4), esterase lipase (C8), α-glucosidase and β-glucosidase, medium activity for valine arylamidase and β-galactosidase, and strong activity for alkaline and acid phosphatases, leucine arylamidase, trypsin, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase. Variable results are obtained for N-acetyl-β-glucosaminidase. Growth occurs in up to 5 % NaCl but not in 10 % NaCl, indicating that the strains are not halophilic but are moderately halotolerant. The G+C content of the strains is 39.9–41.0 mol%. Isolated from microbial mats from lakes Reid, Fryxell and Ace, Antarctica.

The type strain is LMG 21980^T (=DSM 15986^T=R-10710^T).

Acknowledgements

This work was funded by the Bijzonder Onderzoeksfonds (BOF), Universiteit Gent, Belgium. Part of this work was conducted in the framework of the MICROMAT project 'Biodiversity of microbial mats in Antarctica' (project no. BIO4980040), funded by the European Commission under the Biotech Programme.

References

- Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.
- Bowman, J. P., Nichols, C. M. & Gibson, J. A. E. (2003). *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. *Int J Syst Evol Microbiol* **53**, 1343–1355.
- Brettar, I., Christen, R. & Höfle, M. G. (2004). *Belliella baltica* gen. nov., sp. nov., a novel marine bacterium of the *Cytophaga-Flavobacterium-Bacteroides* group isolated from surface water of the central Baltic Sea. *Int J Syst Evol Microbiol* **54**, 65–70.
- Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R., Kersters, K. & Vandamme, P. (1999). Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49**, 405–413.
- Glöckner, F. O., Fuchs, B. M. & Amann, R. (1999). Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl Environ Microbiol* **65**, 3721–3726.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Kirchman, D. L. (2002). The ecology of *Cytophaga-Flavobacterium* in aquatic environments. *FEMS Microbiol Ecol* **39**, 91–100.
- Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003). *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga-Flavobacterium-Bacteroides*. *Int J Syst Evol Microbiol* **53**, 81–85.
- Nedashkovskaya, O. I., Vancanneyt, M., Van Trappen, S. & 7 other authors (2004). Description of *Algoriphagus aquimarinus* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus winogradskyi* sp. nov., from sea water and algae, transfer of *Hongiella halophila* Yi and Chun 2004 to the genus *Algoriphagus* as *Algoriphagus halophilus* comb. nov. and emended descriptions of the genera *Algoriphagus* Bowman *et al.* 2003 and *Hongiella* Yi and Chun 2004. *Int J Syst Evol Microbiol* **54**, 1757–1764.
- Pinhassi, J., Zweifel, U. L. & Hagström, A. (1997). Dominant marine bacterioplankton species found among colony-forming bacteria. *Appl Environ Microbiol* **63**, 3359–3366.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Raes, J. & Van de Peer, Y. (1999). ForCon: a software tool for the conversion of sequence alignments (http://www.ebi.ac.uk/embnet.news/vol6_1/ForCon/body_forcon.html).
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Van de Peer, Y. & De Wachter, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.

Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M. C. & Swings, J. (2002). Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. *Syst Appl Microbiol* **25**, 603–610.

Van Trappen, S., Mergaert, J. & Swings, J. (2003). *Flavobacterium gelidilacus* sp. nov., isolated from microbial mats in Antarctic lakes. *Int J Syst Evol Microbiol* **53**, 1241–1245.

Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., de Lajudie, P., De Vos, P. & Gillis, M. (2001). DNA–DNA hybridization study of *Bradyrhizobium* strains. *Int J Syst Evol Microbiol* **51**, 1315–1322.

Yi, H. & Chun, J. (2004). *Hongiella mannitolivorans* gen. nov., sp. nov., *Hongiella halophila* sp. nov. and *Hongiella ornithinivorans* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **54**, 157–162.