NOTE

***Arthrobacter rhombi* sp. nov., isolated from Greenland halibut (*Reinhardtius hippoglossoides*)**

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Two strains of a hitherto undescribed Gram-positive coryneform bacterium isolated from Greenland halibut (*Reinhardtius hippoglossoides*) were characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing studies demonstrated that the unknown strains constitute a new line within the genus *Arthrobacter*. The nearest relatives of the bacterium from fish were members of the *Arthrobacter nicotianae*/*Arthrobacter sulfureus* group. The unknown bacterium was readily distinguished from these species by phenotypic methods. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Arthrobacter rhombi* sp. nov. The type strain of *Arthrobacter rhombi* is CCUG 38813^T.

Keywords: *Arthrobacter rhombi* sp. nov., taxonomy, phylogeny, 16S rRNA analysis

The genus *Arthrobacter* comprises a group of catalase-positive, strictly aerobic, asporogenous rod-shaped bacteria which display a coryneform morphology. Species of the genus *Arthrobacter* invariably contain cell wall mureins based upon the dibasic amino acid L-lysine (Schleifer & Kandler, 1972) and, phylogenetically, they are members of the Actinomycete branch of the Gram-positive bacteria (Stackebrandt *et al.*, 1997). Amongst species of *Arthrobacter*, two major cell wall murein structural types are recognized, which are designated A3 α and A4 α (for murein nomenclature, see Schleifer & Kandler, 1972). *Arthrobacter globiformis*, the type species of the genus, and most of the other species possess type A3 α in which cross-linkage of murein is by interpeptide bridges consisting of monocarboxylic L-amino acids or glycine, or both. However, a distinct cluster of organisms, viz. *Arthrobacter nicotianae*, *Arthrobacter protophormiae* and *Arthrobacter uratoxydans* referred to as the *Arthrobacter nicotianae* group, possess A4 α mureins in which cross-linkage is by interpeptide bridges containing a dicarboxylic amino acid (type L-Lys-Ala-Glu)

(Stackebrandt *et al.*, 1983). *Arthrobacter sulfureus*, which is phylogenetically associated with the *A. nicotianae* group of species (Stackebrandt *et al.*, 1995; Funke *et al.*, 1996), also contains an A4 α murein but of a slightly modified structure (i.e. type L-Lys-L-Glu) (Stackebrandt *et al.*, 1983). Certain *Micrococcus* species (viz. *Micrococcus luteus* and *Micrococcus lylae*) and *Renibacterium salmoninarum* are phylogenetically intermixed with species of the genus *Arthrobacter* (Stackebrandt *et al.*, 1995). Of these taxa, only *M. lylae* exhibits an A4 α murein (type L-Lys-D-Asp), and comparative 16S rRNA gene sequencing indicates this organism does not have a specific phylogenetic association with the *A. nicotianae* group or *A. sulfureus* (Stackebrandt *et al.*, 1995; Funke *et al.*, 1996). In this article, we report the characteristics of a hitherto unknown *Arthrobacter* species isolated from Greenland halibut which also contains an A4 α cell wall murein. Based on both phenotypic and phylogenetic evidence, a new species, *Arthrobacter rhombi* sp. nov., is described.

Two strains designated F.58.3CB.57 and F.98.3HR.69^T were recovered from organs of Greenland halibut (*Reinhardtius hippoglossoides*). Samples of kidney, spleen and gut were taken from a number of fish from different species including Greenland halibut, captured by deep trawl (300–

Abbreviations: BHA, brain heart infusion agar; CCUG, Culture Collection of the University of Göteborg; RDP, Ribosomal Database Project.

The GenBank accession numbers for the 16S rRNA gene sequences of strains CCUG 38812 and CCUG 38813^T are Y15885 and Y15884, respectively.

800 m) during a fishing expedition carried by the Spanish oceanographic vessel Cornide de Saavedra. The expedition was part of a pluriannual study of the stock populations of fish in the deep waters of the Flemish Cap fishing ground (Newfoundland, NW Atlantic). The temperature of the water at the point of study was 3.5–4.0 °C. Strains F.58.3CB.57 and F.98.3HR.69^T have been deposited in the Culture Collection of the University of Göteborg (CCUG), Sweden, under accession numbers CCUG 38812 and CCUG 38813^T, respectively. The strains were cultured on brain heart infusion agar (BHA) supplemented with 1% (w/v) NaCl at 25 °C. The strains were biochemically characterized by using the API CORYNE and API 50CH systems according to the manufacturer's instructions (API bioMérieux). The cell wall murein structure of strain CCUG 38813^T was determined by the method of Schleifer & Kandler (1972) except that ascending TLC on cellulose sheets (Merck) was used. The G+C content of DNA of strain CCUG 38813^T was determined by thermal denaturation (T_m method; Garvie, 1978). The 16S rRNA genes of the isolates were amplified by PCR and directly sequenced using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches. These sequences and those of other known related strains were retrieved from GenBank or Ribosomal Database Project (RDP) Libraries and aligned with the newly determined sequences using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the program PRETTY from DNADIST (using the Kimura-2 correction parameter) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (200 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). In addition, parsimony analysis (Felsenstein, 1989) was performed on the same data set. The 16S rRNA gene sequences of both strains CCUG 38812 and CCUG 38813^T have been deposited in GenBank under accession numbers Y15885 and Y15884, respectively.

The two isolates were short rods or ovoid in shape and formed single cells. On BHA, strain CCUG 38812 had a whitish-yellow pigmentation, whereas strain CCUG 38813^T had an intense yellow pigmentation. Both strains were Gram-positive obligate aerobes. They grew in 1 and 10% (w/v) NaCl, at 4 and 30 °C. Using the API 50CH system, both isolates utilized glycerol, galactose, D-glucose, D-fructose, D-mannose, mannitol, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, β -gentiobiose, D-turanose, gluconate and D-arabitol as sole carbon sources (Table 1). Both strains failed to

Table 1. Biochemical characteristics of *Arthrobacter rhombi* sp. nov. and its closest relatives

Strains: 1, *A. rhombi* CCUG 38812, 38813^T; 2, *A. sulfureus* NCIMB 10355^T; 3, *A. uratoxydans* DSM 20647^T; 4, *A. protophormiae* NCIMB 12765^T; 5, *A. nicotianae* DSM 20123^T.

Characteristic	1	2	3	4	5
Activities:*					
Nitrate reduction	–	–	+	+	+
Pyrazinamidase	–	+	+	+	+
β -Galactosidase	+	–	–	–	–
Urease	–	+	+	–	–
Assimilation of:†					
Amygdalin	+	–	–	–	+
Arbutin	+	–	–	–	+
Cellobiose	+	–	–	+	+
D-Arabitol	+	+	–	–	+
D-Mannose	+	–	–	–	–
D-Turanose	+	–	–	–	–
D-Xylose	–	–	–	–	+
Galactose	+	–	–	–‡	+
Glycerol	+	–	+§	+	+
Inositol	–	+	–	–	–
L-Arabinose	–	–	–	–	+
Maltose	+	+	–	+	+
Mannitol	+	+	–	–	–
Melibiose	+	–	–	–	–
Rhamnose	v	–	–‡	–	–
Ribose	–	–	+§	–	+
Salicin	+	–	–	–	+
Starch	–	–	–	–	+
Sucrose	+	–	–	–	–
Trehalose	+	–	–	–	–
Xylitol	–	+	–	–	–
β -Gentiobiose	+	–	–	–	+
N-Acetylglucosamine	–	–	+	–	–
5-Ketogluconate	–	+	–	+	–

* API CORYNE system.

† Carbohydrate assimilation reactions: API 50CH and AUX Medium, incubated at 25 °C (Funke & Carlotti, 1994); +, positive; –, negative; v, variable.

‡ Positive in the study of Hou *et al.* (1998).

§ Negative in the study of Hou *et al.* (1998).

assimilate erythritol, D-arabinose, L-arabinose, ribose, D-xylose, adonitol, methyl β -xyloside, L-sorbose, dulcitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, inositol, N-acetylglucosamine, inulin, melizitol, D-raffinose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate and 5-ketogluconate as sole carbon sources. Rhamnose was utilized by strain CCUG 38812 but not by CCUG 38813^T. Using the API CORYNE system, both isolates showed β -galactosidase activity; no activity for pyrazinamidase or urease was detected. Neither strain reduced nitrate. An examination of the

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Arthrobacter rhombi* sp. nov. within the genus *Arthrobacter*. The tree constructed using the neighbour-joining method was based on a comparison of approx. 1320 nucleotides. Bootstrap values, expressed as a percentage of 200 replications, are given at branching points. Bar, 1% sequence divergence. With the exception of *A. rhombi* CCUG 38812, all other strains are type strains; accession numbers are given in parentheses.

cell wall of strain CCUG 38813^T revealed the presence of an A4 α murein (type L-Lys-L-Ala-D-Glu). Within the coryneform group of bacteria, this murein type is found within the *A. nicotianae* group (viz. *A. nicotianae*, *A. protophormiae*, *A. uratoxydans*, *Arthrobacter creatinolyticus*) (Schleifer & Kandler, 1972; Stackebrandt *et al.*, 1983; Hou *et al.*, 1998). *A. sulfureus* also contains an A4 α murein but this is of the L-Lys-L-Glu type (Schleifer & Kandler, 1972). To investigate the phylogenetic affinity between the two isolates, and their relationship with other coryneform taxa, comparative 16S rRNA gene sequence analyses were performed. The almost complete gene sequence (> 1400 nucleotides) of the two strains was determined and pairwise analysis revealed 99.9% sequence similarity (corresponding to a single base difference) thereby showing their high phylogenetic relatedness. Sequence searches of GenBank and RDP Libraries revealed that the unknown bacterium was phylogenetically most closely associated with members of the genus *Arthrobacter* (data not shown). Highest sequence relatedness values were shown to *A. sulfureus* (96.7% similarity), *A. nicotianae* (96.6% similarity), *A. protophormiae* (95.6% similarity) and *A. uratoxydans* (95.8% similarity). A tree constructed by the neighbour-joining method depicting phylogenetic affinities of the unknown bacterium is shown in Fig. 1. From the branching pattern of the tree, the nearest relative of the unknown bacterium was *A. sulfureus*. The clustering together of these organisms occurred in 93% of 200 tree replications. The next nearest relatives of the aforementioned taxa corresponded to *A. nicotianae*, *A. uratoxydans* and *A. protophormiae*.

The yellow-pigmented coryneform isolates from halibut phenotypically resembled the genus *Arthrobacter*. In particular, the presence of an A4 α type L-Lys-L-Ala-D-Glu murein indicated that the unknown isolates were members of the *A. nicotianae* group of organisms. The results of 16S rRNA gene sequencing confirmed the placement of the fish bacterium within the genus *Arthrobacter* but showed *A. sulfureus* to be its nearest relative (Fig. 1). The association between the unknown bacterium and *A. sulfureus* was statistically highly significant. The *A. nicotianae* group was, however, the next nearest relative of the unknown bacterium. Sequence divergence values between the fish bacterium and *A. sulfureus* and the *A. nicotianae* group were > 3% and unequivocally demonstrated its separate species status. In this context, it is pertinent to note that the levels of sequence divergence shown between the fish bacterium and the aforementioned taxa are significantly greater than those shown between several genomically distinct *Arthrobacter* species. In addition to differing from *A. sulfureus* in murein composition, the unknown bacterium was readily differentiated from this species in assimilating a larger number of carbohydrates and by its positive reaction with β -galactosidase and negative reactions with pyrazinamidase and urease. Similarly, the bacterium from halibut was readily distinguished from species of the *A. nicotianae* group by its distinct carbohydrate assimilation reactions and enzymic profile (see Table 1). Recently, a new member of the *A. nicotianae* group, *A. creatinolyticus* (isolated from human urine) was described (Hou *et al.*, 1998). The closest relative of the latter species is *A. protophormiae* (Hou *et al.*, 1998).

16S rRNA pairwise analysis between the unknown bacterium (accession no. Y15884) from fish and *A. creatinolyticus* (accession no. D88212) clearly showed that these taxa are phylogenetically quite separate species (approx. 10% sequence divergence). In view of the distinct biochemical features and separate 16S rRNA subline formed by the unknown *Arthrobacter* from fish, we propose that it be designated a new species, *Arthrobacter rhombi* sp. nov.

Description of *Arthrobacter rhombi* sp. nov.

Arthrobacter rhombi (rhom'bi. L. masc. N. *rhombus* flatfish; *rhombi* of flatfish).

Cells are Gram-positive, short rods and cocci. They are non-spore-forming and non-motile. On BHA, smooth convex colonies which are yellowish or whitish in colour and with a diameter of approx. 1 mm are formed after 48 h. Growth occurs in 1% (w/v) and 10% (w/v) NaCl and at 4 and 30 °C. They are strictly aerobic and catalase- and oxidase-positive. By using the API CORYNE system, positive reactions for β -galactosidase and esculinase are observed. Negative reactions are obtained for nitrate reductase, pyrazinamidase, pyrrolidonyl arylamidase, urease, β -glucuronidase, *N*-acetyl- β -glucosaminidase and gelatinase. Using the API 50CH system, the following substrates are used as sole carbon sources: glycerol, galactose, D-glucose, D-fructose, D-mannose, mannitol, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, β -gentiobiose, D-turanose, gluconate and D-arabitol. Erythritol, D-arabinose, L-arabinose, ribose, D-xylose, adonitol, methyl β -xyloside, L-sorbose, dulcitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, inositol, *N*-acetyl- β -glucosamine, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-keto-gluconate and 5-ketogluconate are not utilized. The cell wall murein type is A4 α (L-Lys-L-Ala-D-Glu). The G+C content of DNA is 61 mol% (T_m method). Isolated from organs of Greenland halibut (*Reinhardtius hippoglossoides*) in the deep waters of the Flemish Cap fishing ground (Newfoundland, NW Atlantic). The type strain, CCUG 38813^T, was isolated from the spleen of *Reinhardtius hippoglossoides*.

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