NOTE

***Abiotrophia balaenopterae* sp. nov., isolated from the minke whale (*Balaenoptera acutorostrata*)**

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Phenotypic and phylogenetic studies were performed on a hitherto undescribed micro-organism isolated from a minke whale (*Balaenoptera acutorostrata*). Comparative 16S rRNA gene sequencing studies demonstrated that the unknown strain constituted a new subtype close to, but distinct from, *Abiotrophia adiacens* and *Abiotrophia elegans*. The unknown bacterium was readily distinguished from these two *Abiotrophia* species by biochemical tests and electrophoretic analysis of whole-cell proteins. On the basis of phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Abiotrophia balaenopterae* sp. nov., the type strain of which is M1975/96/1^T (= CCUG 37380^T).

Keywords: *Abiotrophia balaenopterae* sp. nov., phylogeny, taxonomy, 16S rRNA

Until recently, the genus *Abiotrophia* included two species, *Abiotrophia adiacens* and *Abiotrophia defectiva* (Kawamura *et al.*, 1995). These organisms were originally described by Frenkel & Hirsch (1961) as a new type of viridans-group streptococci that exhibited satellitism around the colonies of other bacteria. Historically, these bacteria have been referred to by a variety of terms, such as nutritionally variant streptococci (Cooksey *et al.*, 1979) and pyridoxal-dependent streptococci (Roberts *et al.*, 1979), because of their fastidious nutritional requirements. Bouvet *et al.* (1989) showed the presence of two distinct genetic populations amongst the nutritionally variant streptococci by using DNA–DNA pairing and named the species *Streptococcus adiacens* and *Streptococcus defectivus*. Subsequent 16S rRNA sequencing studies by Kawamura *et al.* (1995) showed that *S. adiacens* and *S. defectivus* were phylogenetically remote from authentic streptococci and these authors proposed the genus *Abiotrophia* to accommodate these species. Recently, a third species of the genus, *Abiotrophia elegans*, was described from a patient with endocarditis (Roggenkamp *et al.*, 1998).

Abiotrophiae colonize the oral cavity and are residents of the human intestinal and genitourinary tracts (Ruoff, 1991). They cause sepsis and bacteraemia and

are responsible for a significant proportion of cases of infective endocarditis (Bouvet, 1995; Roberts *et al.*, 1979). The identification of *Abiotrophia* spp. and related organisms is often problematic. They usually grow poorly in media used routinely for streptococci, e.g. blood agar, and usually require supplements such as pyridoxal. In addition to their fastidiousness, aberrant morphological and staining characteristics and atypical biochemical characteristics further complicate their recognition and reliable identification. *A. adiacens* and *A. defectiva* are, however, phylogenetically very distinct species and possess highly characteristic 16S rRNA genes (Kawamura *et al.*, 1995). Ohara-Nemoto *et al.* (1997) recently reported the use of this marker as a reliable means for identifying *A. adiacens* and *A. defectiva*. In the course of a study of Gram-positive, catalase-negative cocci associated with sea mammals, 16S rRNA gene sequencing has been used to characterize a hitherto unknown *Abiotrophia*-like bacterium phylogenetically. On the basis of the results of a polyphasic taxonomic study, a new species, *Abiotrophia balaenopterae* sp. nov., is described.

Strain M1975/96/1^T was isolated following a post-mortem examination from a minke whale beached on the north-western coast of Scotland. The unknown coccus was recovered as part of the dominant flora of a mixed culture from a 'heavy growth' found within the lungs of the whale, as well as being recovered in

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 37380^T is Y16547.

mixed culture from the spleen. The organism was the sole isolate from both the liver and kidneys. It was, however, not possible to draw conclusions regarding the possible clinical significance of the unknown isolate. The strain has been deposited in the Culture Collection of the University of Göteborg (CCUG) Sweden under the collection number CCUG 37380^T.

The unidentified organism was cultured on Columbia agar supplemented with 5% defibrinated horse blood (Oxoid; catalogue no. P0122A) at 37 °C in air plus 5% CO₂. The strain was characterized biochemically by using the API Rapid ID32S and API ZYM systems according to the manufacturer's instructions (bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). For densitometric analysis, normalization and interpretation of protein patterns the GELCOMP GCW 3.0 software package (Applied Maths) was used. The cell wall murein structure and G+C content of DNA of strain CCUG 37380^T were determined as described by Schleifer & Kandler (1972) and Garvie (1978), respectively. The 16S rRNA genes of the isolate were amplified by PCR and sequenced directly by using a *Taq* dye-Deoxy terminator cycle sequencing kit (ABI) and an automatic DNA sequencer (model 373A; ABI). The closest known relatives of the new isolate were determined by performing database searches. These sequences and those of other known related strains were retrieved from GenBank or the Ribosomal Database Project (RDP) library and aligned with the newly determined sequence by using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated by using the programs PRETTY and 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 (500 replications) with the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The unknown bacterium from minke whale was a Gram-positive coccus that occurred as single cells, in pairs or in short chains. The isolate was catalase-negative, facultatively anaerobic and produced small pinhead colonies on Columbia agar supplemented with 5% horse blood grown at 37 °C in air plus 5% CO₂. Neither the addition of pyridoxal to the blood agar nor satellitism was required to support growth. The strain was non-haemolytic. The isolate produced acid from glucose, maltose, pullulan and trehalose. Acid was not produced from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, D-raffinose, D-ribose, sorbitol, sucrose, tagatose or D-xylose. With the commercial API systems, the isolate showed *N*-acetylglucosaminidase, arginine dihydrolase, ester lipase (C₈), leucine arylamidase, pyroglutamic acid arylamidase (weak reaction) and urease (weak re-

Fig. 1. Similarity dendrogram based on whole-cell protein pattern of *Abiotrophia balaenopterae* sp. nov. and related species. Levels of correlation are expressed as percentage similarities for convenience. Duplicate type strains of *Gemella morbillorum* and *Aerococcus urinae* held by CCUG are shown.

action) activities. No activity of acid phosphatase, alkaline phosphatase, alanyl-phenylalanine-proline arylamidase, α -galactosidase, β -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, β -glucuronidase, glycyl-tryptophan arylamidase, β -mannosidase, chymotrypsin, α -fucosidase or pyrazinamidase was detected. Aesculin was hydrolysed but hippurate and gelatin were not. The isolate did not reduce nitrate and acetoin was not produced. PAGE analysis of whole-cell proteins showed the unknown isolate to be separate from all other Gram-positive, catalase-negative reference organisms examined, including *Abiotrophia* spp., *Carnobacterium* spp., *Facklamia hominis* and *Globicatella sanguinis* (Fig. 1). Strain CCUG 37380^T possessed a murein based on L-Orn-D-Asp (type A4 β) (nomenclature of Schleifer & Kandler, 1972) and a DNA G+C content of 37 mol%. In order to assess the genealogical affinity between the unknown organism and other Gram-positive, catalase-negative taxa, comparative 16S rRNA gene sequence analyses were performed. The almost complete gene sequence (> 1400 nucleotides) of the unknown coccus was

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Abiotrophia balaenopterae* sp. nov. and some other low G+C content, Gram-positive bacteria. The tree, constructed by using the neighbour-joining method, was based on a comparison of approx. 1320 nucleotides. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points.

determined. Sequence searches of GenBank and RDP libraries revealed that the unknown bacterium was associated most closely phylogenetically with the lactic acid group of bacteria. A tree, constructed by neighbour-joining, depicting the phylogenetic affinity of the unknown coccus is shown in Fig. 2. From the treeing analysis, it was evident that the closest relatives of the unknown coccus were *A. adiacens* and *A. elegans*. Pairwise comparisons showed that the 16S rRNA of the unknown organism contained 56 and 72 differences (based on 1370 positions) from *A. adiacens* and *A. elegans*, respectively, corresponding to > 3% sequence divergence.

The unknown isolate from the minke whale clearly belongs to a hitherto unrecognized, Gram-positive, catalase-negative species within the lactic acid group of bacteria. From the 16S rRNA sequence analysis, it

is evident that the coccus has a close phylogenetic relationship with *A. adiacens* and *A. elegans*. Sequence divergence values of approx. 3.5 and 5%, respectively, from these two species demonstrate, however, that this relationship is that of phylogenetically closely related, but nevertheless quite separate, species. Support for the separate specific status of the unknown bacterium from minke whale comes from its possession of an L-Orn-D-Asp-based, directly cross-linked murein (type A4β). This was an unexpected finding, as this murein structure is very unusual among coccus-shaped taxa and has, to our knowledge, been described only for *Sporosarcina halophila* and *Peptococcus glycinophilus*. This trait not only distinguishes the unknown bacterium from previously described *Abiotrophia* species, but also from all other low G+C-content lactic acid bacteria. Examples of characteristics which serve to distinguish the unknown coccus from *A. adiacens*, *A. defectiva* and *A. elegans* are outlined in Table 1.

Description of *Abiotrophia balaenopterae* sp. nov.

Abiotrophia balaenopterae (bal.aen.op'ter.ae. M.L. fem. n. *balaenopterae* pertaining to minke whale, *Balaenoptera acutorostrata*, from which the organism was isolated).

Cells are Gram-positive cocci occurring as single cells, in pairs or in short chains. Cells are non-spore-forming and non-motile. Tiny colonies up to 0.2 mm in diameter are formed on Columbia agar supplemented with 5% horse blood at 37 °C. Neither pyridoxal hydrochloride nor satellitism is required to support growth. Haemolysis is not observed. Facultatively anaerobic and catalase-negative. Acid is produced from glucose, maltose, pullulan and trehalose. Acid is not produced from L-arabinose, D-arabitol, cyclo-dextrin, glycogen, lactose, mannitol, melibiose, mel-ezitose, D-raffinose, D-ribose, sucrose, sorbitol, tagatose or D-xylose. Arginine dihydrolase, pyroglutamic

Table 1. Characteristics that differentiate *A. balaenopterae* sp. nov. from *A. adiacens*, *A. defectiva* and *A. elegans*

Character	<i>A. balaenopterae</i>	<i>A. defectiva</i>	<i>A. adiacens</i>	<i>A. elegans</i>
Production of acid from:				
Pullulan	+	v	—	—
Sucrose	—	+	+	+
Tagatose	—	—	+	—
Trehalose	+	v	—	—
Hydrolysis of:				
Hippurate	—	—	—	v
Production of:				
Arginine dihydrolase	+	—	—	+
α-Galactosidase	—	+	—	—
β-Glucuronidase	—	—	+	—
Murein type	A4β	A1α	A3α	ND

v, Variable; ND, not determined.

acid arylamidase (weak reaction), *N*-acetylglucosaminidase, ester lipase (C_8), leucine arylamidase and urease (weak reaction) activity is detected. Alkaline phosphatase, acid phosphatase, alanyl-phenylalanine-proline arylamidase, α -galactosidase, β -galactosidase, β -galacturonidase, β -glucuronidase, glycyl-tryptophan arylamidase, α -mannosidase, β -mannosidase, chymotrypsin, trypsin, α -fucosidase and pyrazinamidase activity is not detected. Aesculin is hydrolysed. Hippurate and gelatin are not hydrolysed. Nitrate is not reduced. The cell wall contains an L-Orn-D-Asp-based, directly cross-linked murein (type A4 β). The G+C content of DNA of the type strain is 37 mol% (T_m). As determined by 16S rRNA gene sequence analysis, the new species belongs to the lactic acid group of bacteria with low DNA G+C contents, and is phylogenetically closely related to *A. adiacens* and *A. elegans*, but may be distinguished from the latter two species using traits shown in Table 1. Isolated from minke whale. Habitat not known. The type strain is CCUG 37380^T.

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