NOTE

***Arthrobacter nasiphocae* sp. nov., from the common seal (*Phoca vitulina*)**

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An unknown Gram-positive, catalase-positive, strictly aerobic, rod-shaped bacterium was isolated from the nasal cavities of two common seals. Chemical analysis revealed the presence in the bacterium of a hitherto unknown cell-wall murein [type: L-Lys-L-Ala₂-Gly₂₋₃-L-Ala (Gly)]. Comparative 16S rRNA gene sequencing showed that the unidentified rod was related to the *Arthrobacter* group of organisms, although sequence divergence values of > 3% from established members of this genus indicated that it represents a novel species. On the basis of phenotypic and phylogenetic considerations, it is proposed that the unknown bacterium from seals (*Phoca vitulina*) be classified as a novel species, *Arthrobacter nasiphocae* sp. nov. The type strain of *Arthrobacter nasiphocae* is CCUG 42953^T.

Keywords: *Arthrobacter nasiphocae*, seal, taxonomy, phylogeny, 16S rRNA

The genus *Arthrobacter* consists of a group of catalase-positive, strictly aerobic, asporogenous, rod-shaped micro-organisms that exhibit a coryneform morphology. Phylogenetically, the genus *Arthrobacter* belongs to the *Actinomyces* branch of the Gram-positive bacteria and is closely related to micrococci, *Kocuria* species, *Nesterenkonia halobia* and *Renibacterium salmoninarum* (Stackebrandt *et al.*, 1995, 1997). The genus *Arthrobacter* is phenotypically heterogeneous, and over 25 species are currently recognized. Amongst species of *Arthrobacter*, two major cell-wall murein structural variations are known, designated A3 α and A4 α (Schleifer & Kandler, 1972). *Arthrobacter globiformis* (the type species of the genus) and the majority of other *Arthrobacter* species contain the A3 α wall variation, in which murein cross-linkage is by interpeptide bridges consisting of monocarboxylic L-amino acids or glycine, or both. By contrast, several other *Arthrobacter* species, referred to as the *Arthrobacter nicotianae* and *Arthrobacter sulfureus* groups, possess the A4 α wall variation, in which cross-linkage is achieved by interpeptide bridges containing a dicarboxylic acid (type L-Lys-Ala-Glu and type L-Lys-Glu). During the course of a study of taxonomically problematic members of the *Actinobacteria* from

animal sources, we have characterized an unusual *Arthrobacter*-like bacterium, from seals (*Phoca vitulina*), which contains a hitherto undescribed A3 α wall murein type. On the basis of the results of a polyphasic taxonomic study, we propose that the unknown bacterium from seals be classified as a novel species of the genus *Arthrobacter*, *Arthrobacter nasiphocae* sp. nov.

Strains M597/99/9 and M597/99/10^T were isolated from nasal swabs taken from two common seals housed in a rehabilitation centre. The strains were isolated in mixed culture with *Corynebacterium phocae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and coagulase-negative staphylococci. The unidentified strains were cultured on Columbia blood-agar base supplemented with 5% defibrinated horse blood at 37 °C, in air plus 5% CO₂. The strains were biochemically characterized by using the API CORYNE and API ZYM systems according to the manufacturer's instructions (API bioMérieux). Growth in NaCl was determined in nutrient broth (Difco). PAGE analysis of whole-cell proteins was determined as described by Pot *et al.* (1994) and numerical analyses were performed by using the GELCOMP 3.0 software package (Applied Maths). Cell-wall murein was prepared by mechanical disruption of cells, and acid hydrolysates were analysed as described by Schleifer & Kandler (1972) except that ascending TLC with cellulose sheets was used. The menaquinone com-

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain M597/99/10^T (= CCUG 42953^T = CIP 107054^T) is AJ292364.

Fig. 1. Unrooted tree based on 16S rRNA, showing the phylogenetic relationships of *Arthrobacter nasiphocae* sp. nov. Bar, 1% sequence divergence.

position was determined as described by Collins (1994). The 16S rRNA genes of the isolates were amplified by a PCR and sequenced directly using a *Taq* Dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the novel isolates were determined by performing database searches. These sequences and those of other known related strains were retrieved from the GenBank or Ribosomal Database Project libraries and aligned with the newly determined sequences using the program DNATOOLS (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated 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 by using the program NEIGHBOR, and the stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The two unidentified isolates recovered from the nasal swabs consisted of Gram-positive short rods that were strictly aerobic and catalase-positive. The organism grew with or without blood. Using the API CORYNE system, neither of the isolates produced acid from glucose or the other carbohydrates tested. The two isolates displayed identical enzyme profiles with the

API CORYNE and ZYM systems: alkaline phosphatase, acid phosphatase, α -glucosidase, esterase C₄ (weak), phosphoamidase, pyrazinamidase, pyroglutamic acid arylamidase and leucine arylamidase activities were detected, but no activity was found for cystine arylamidase, chymotrypsin, ester lipase C₈, α -fucosidase, α -galactosidase, β -galactosidase, β -glucosidase, β -glucuronidase, lipase C₁₄, α -mannosidase, *N*-acetyl- β -glucosaminidase, valine arylamidase, urease or trypsin. The two strains hydrolysed gelatin but did not reduce nitrate. In terms of their overall morphological and biochemical characteristics, the unidentified rod-shaped micro-organisms closely resembled members of the genus *Arthrobacter* but did not appear to correspond to any recognized species of this genus. Cell-wall analysis was conducted on strain M597/99/10^T and revealed the presence of an A3 α murein type: L-Lys-L-Ala₂-Gly₂₋₃-L-Ala (Gly). Hydrolysis of dinitrophenylated walls revealed DNP-Ala and DNP-Gly (DNP, dinitrophenyl), indicating that both alanine and glycine constitute the N-terminal amino acids of the interpeptide bridge. This murein type has not been reported in any other organism to date. The menaquinone composition of strain M597/99/10^T was determined and shown to correspond to 56% MK-9(H₂) and 44% MK-8(H₂). PAGE analysis of whole-cell proteins showed that the unknown rod-shaped seal bacterium was distinct from all other reference *Arthrobacter* species (data not shown). According to PAGE analysis, the species nearest to the

unknown bacterium corresponded to *Arthrobacter cummingsii* and *Arthrobacter albus*, joining at a correlation level of 60 %, other species being related more distantly (correlation levels of < 50 %).

To investigate the phylogenetic affinity between the unidentified isolates and to ascertain their relationships with other taxa, comparative 16S rRNA gene sequencing was performed. The almost complete gene sequences of the two isolates were determined and pairwise analysis showed them to be identical, thereby demonstrating their close genotypic relatedness. Sequence database searches revealed that the unknown bacterium was related most closely to members of the genus *Arthrobacter* (approximate range 94–96.5 % sequence similarity). A tree constructed using the neighbour-joining method depicting the phylogenetic placement of the unidentified bacterium (as exemplified by strain M597/99/10^T) is shown in Fig. 1 and shows that it represents a novel species within the genus. The unknown seal bacterium clustered loosely with *Arthrobacter woluwensis*, although bootstrap resampling showed that this association was not statistically significant.

The results of the polyphasic taxonomic analysis clearly show that the unknown rod-shaped seal bacterium represents a hitherto unrecognized species within the genus *Arthrobacter*. Phylogenetically, the bacterium formed a distinct subline within the genus and did not show a specific or close affinity with any other *Arthrobacter* species. Furthermore, sequence divergence values of > 3 % from other members of the genus demonstrate unequivocally that the unidentified seal bacterium represents a novel species (Stackebrandt & Goebel, 1994). Support for the distinctiveness of the unknown species was also very evident from whole-cell protein profiling, cell-wall analyses and its biochemical characteristics. In particular, the presence of a unique A3 α cell-wall murein [type: L-Lys–L-Ala₂–Gly₂₋₃–L-Ala (Gly)] and an API CORYNE profile of 6112004 serve to distinguish the novel seal bacterium from all recognized *Arthrobacter* species. Therefore, on the basis of both phenotypic and phylogenetic evidence, we propose that the unknown seal bacterium should be classified within the genus *Arthrobacter*, as *Arthrobacter nasiphocae* sp. nov.

Description of *Arthrobacter nasiphocae* sp. nov.

Arthrobacter nasiphocae (na.si.pho'cae. L. masc. n. *nasus* nose; Gr. n. *phoca* seal; N.L. gen. n. *nasiphocae* of the nose of a seal).

The cells are Gram-positive, non-spore-forming, irregular-shaped rods; coccoid forms may be observed. Colonies are circular, entire, convex and approxi-

mately 1 mm in diameter after 24 h at 37 °C on blood agar. Colonies are greyish-white in colour and are non-haemolytic on blood agar. Strictly aerobic and catalase-positive. Growth is produced at 25 and 42 °C. Grows in broth containing 5 % NaCl but not in 10 % NaCl. Acid is not produced from glucose, glycogen, lactose, mannitol, maltose, ribose, sucrose or D-xylose. Activity is detected for alkaline phosphatase, acid phosphatase, α -glucosidase, esterase C₄ (weak), phosphoamidase, pyrazinamidase, pyroglutamic acid arylamidase and leucine arylamidase. No activity is detected for cystine arylamidase, chymotrypsin, ester lipase C₈, α -fucosidase, α -galactosidase, β -galactosidase, β -glucosidase, β -glucuronidase, lipase C₁₄, α -mannosidase, N-acetyl- β -glucosaminidase, valine arylamidase, urease or trypsin. Gelatin and hippurate are hydrolysed but aesculin and starch are not. Nitrate is not reduced. Acetoin is not produced. Cell-wall murein is based on L-lysine variation A3 α [type: L-Lys–L-Ala₂–Gly₂₋₃–L-Ala (Gly)]. The major menaquinones are MK-9(H₂) and MK-8(H₂). The G + C content of the DNA is 65 mol %. Isolated from the nose of the common seal (*Phoca vitulina*). Habitat unknown. The type strain is M597/99/10^T (= CCUG 42953^T = CIP 107054^T).

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