NOTE

***Facklamia miroungae* sp. nov., from a juvenile southern elephant seal (*Mirounga leonina*)**

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An unusual Gram-positive, catalase-negative, facultatively anaerobic, coccus-shaped organism that originated from a juvenile elephant seal was characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing showed that the unknown coccus represents a new subspecies within the genus *Facklamia*. The unknown strain was readily distinguishable from all currently recognized species of the genus *Facklamia* (*Facklamia hominis*, *Facklamia languida*, *Facklamia ignava*, *Facklamia sourekii* and *Facklamia tabacinasalis*) by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Facklamia miroungae* sp. nov. The type strain of *F. miroungae* is CCUG 42728^T (= CIP 106764^T). *F. miroungae* is the first member of the genus *Facklamia* to be isolated from an animal other than man.

Keywords: taxonomy, phylogeny, *Facklamia miroungae*, 16S rRNA

The genus *Facklamia* was proposed in order to accommodate a phylogenetically distinct group of Gram-positive, catalase-negative, chain-forming, coccus-shaped organisms encountered in human clinical specimens (Collins *et al.*, 1997). Originally, the genus was monospecific, containing only the species *Facklamia hominis*. More recently, three further species from human clinical samples have been assigned to the genus, *Facklamia ignava* (Collins *et al.*, 1998), *Facklamia sourekii* (Collins *et al.*, 1999a) and *Facklamia languida* (Lawson *et al.*, 1999), along with *Facklamia tabacinasalis*, a biochemically unreactive species isolated as a contaminant of powdered tobacco (Collins *et al.*, 1999b). In this article, we report the results of a polyphasic taxonomic study on an unusual *Facklamia*-like organism originating from a juvenile southern elephant seal (*Mirounga leonina*). On the basis of the findings presented, we consider that the unknown coccus represents yet another species of the genus *Facklamia*, for which the name *Facklamia miroungae* sp. nov. is proposed.

Strain A/G13/99/2^T was isolated from a nasal swab of a juvenile elephant seal, as part of a study carried out by the British Antarctic Survey in the South Orkneys

in 1993. The strain could not be identified by conventional morphological and biochemical testing by SAC Veterinary Sciences Division, Inverness, UK. The unknown coccus has been deposited in the Culture Collection of the University of Göteborg under accession number CCUG 42728^T. Strain A/G13/99/2^T was cultured on Columbia agar (Oxoid) supplemented with 5% horse blood 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 (API bioMérieux). The 16S rRNA gene of the isolate was amplified by PCR and sequenced directly using a *Taq* Dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automated DNA sequencer (model 373A, Applied Biosystems). The closest known relatives of the unknown isolate were determined by performing database searches. A phylogenetic tree was constructed using the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (350 replications), using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). The software package GELCOMPAR GCW version 3.0 (Applied Maths) was used for densitometric analysis, normalization and interpretation of protein patterns. The profiles

The GenBank/EMBL accession number for the 16S rRNA sequence of CCUG 42728^T is AJ277381.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *F. miroungae* sp. nov. and its close relatives. The tree, constructed using the neighbour-joining method, was based on a comparison of approx. 1300 nucleotides. Bootstrap values, expressed as percentages of 350 replications, are given at branching points. Bar, 1% sequence divergence.

generated were compared with a comprehensive database maintained by the CCUG.

The unknown bacterium from a juvenile seal was ovoid in shape and cells were arranged in pairs or short chains. The isolate was Gram-positive, non-spore-forming, catalase-negative and facultatively anaerobic. Growth was not enhanced by CO₂. The isolate grew at 25 and 42 °C and growth was optimal at 37 °C. It was also moderately halotolerant, growing in 5% NaCl. Using commercial API systems, the isolate produced acid from glucose and trehalose, but was unreactive for other sugars tested. Enzymically, the unknown coccus showed activity for arginine dihydrolase, alanine-phenylalanine-proline arylamidase and urease. Weak activity was observed for valine arylamidase, cysteine arylamidase, chymotrypsin, phosphoaminidase, acid phosphatase and glycyl-tryptophan arylamidase. The organism was Voges-Proskauer-negative and did not hydrolyse aesculin, hippurate or gelatin. It did not reduce nitrate to nitrite. To assess the phylogenetic position of the unknown coccus, its 16S rRNA gene sequence was determined (> 1415 nucleotides). Sequence searches of GenBank/EMBL revealed that the unknown bacterium was

phylogenetically most closely related to members of the genus *Facklamia*. Highest sequence similarity was shown to *F. languida* (96.8% sequence similarity based on a comparison of 1394 bp) and *F. hominis* (96.3% sequence similarity based on a comparison of 1473 bp). Other *Facklamia* species and *Ignavigranum ruoffiae* displayed significantly lower sequence relatedness. Treeing analysis confirmed the affinity of the unknown bacterium with members of the genus *Facklamia*. Strain CCUG 42728^T formed a distinct subline within the *F. hominis*–*F. languida* subcluster of the genus (Fig. 1). The clustering of the three aforementioned taxa was statistically significant (100% in 350 replications). PAGE analysis of whole-cell proteins confirmed that the unknown coccus was phenotypically distinct from other members of the genus *Facklamia* and all other reference species examined. The closest species to the unidentified coccus was *F. tabacinasalis*, joining at a correlation level of 70%. Other *Facklamia* species were more distantly related (data not shown).

It is evident from both phenotypic and phylogenetic studies that the unidentified isolate recovered from a nasal swab of a juvenile southern elephant seal represents a hitherto unknown *Facklamia* species. Both sequence divergence values and treeing analysis show that the bacterium represents a new subline within the genus *Facklamia*. The nearest phylogenetic relatives of the unknown coccus correspond to *F. hominis* and *F. languida*, although sequence divergence values of 3.7 and 3.2% from these taxa show unequivocally that the unidentified seal bacterium represents a previously unknown species. From the analysis performed, it is apparent that the genus *Facklamia* is not monophyletic and is in fact intermixed with *Ignavigranum ruoffiae*. However, since the seal isolate is phylogenetically highly related to the type species of the genus *Facklamia* (*F. hominis*), the unknown bacterium is clearly an authentic member of this genus. Therefore, on the basis of our phylogenetic findings

Table 1. Tests that are useful in distinguishing *F. miroungae* sp. nov. from some other *Facklamia* species and *Ignavigranum ruoffiae*

Tests were performed under identical conditions, as described in the text, using the API Rapid ID32S system. Results are scored as: –, negative; +, positive; v, variable; +^w, strains positive or weakly positive; +⁽⁻⁾, most strains positive, but some negative.

Characteristic	<i>F. miroungae</i>	<i>F. hominis</i>	<i>F. ignava</i>	<i>F. sourekii</i>	<i>F. languida</i>	<i>I. ruoffiae</i>
Acid from:						
D-Arabitol	–	–	–	+ ⁽⁻⁾	–	–
Sucrose	–	–	–	+	–	v
Trehalose	+	–	+ ^w	v	+	–
Production of:						
Arginine dihydrolase	+	+	–	–	–	+
Alanine-phenylalanine-proline arylamidase	+	+	+	–	–	–
α-Galactosidase	–	+	–	–	–	–
β-Galactosidase	–	+	–	–	–	–
Urease	+	v	–	–	–	+
Hydrolysis of hippurate	–	+	+	+	–	–

and the phenotypic distinctiveness of the seal bacterium, we propose that the unknown bacterium be classified as a new species of the genus *Facklamia*, *Facklamia miroungae* sp. nov. Tests useful for distinguishing *F. miroungae* from other *Facklamia* species are shown in Table 1.

Description of *Facklamia miroungae* sp. nov.

Facklamia miroungae (mi.roung'ae. N.L. gen. n. *miroungae* of *Mirounga*, named because the organism was isolated from the southern elephant seal, *Mirounga leonina*).

Cells are Gram-positive, ovoid in shape (approx. 0.8–0.9 µm in diameter) and occur in pairs or short chains. Cells are asporogenous and non-motile. Colonies on blood agar are γ -haemolytic, small (< 0.5 mm in diameter after 24 h), circular, entire, shiny, convex and grey in colour. Facultatively anaerobic and catalase-negative. Grows in 5% NaCl. Grows at 25 and 42 °C. Acid is produced from glucose and trehalose, but not from mannitol, maltose, sorbitol, lactose, raffinose, glycogen, pullulan, melibiose, melzitose, L-arabinose, D-arabitol, tagatose, methyl β -D-glucopyranoside, cyclodextrin or sucrose. Activity is detected for arginine dihydrolase, alanine-phenylalanine-proline arylamidase, pyroglutamic acid arylamidase, leucine arylamidase and urease. Weak activity is observed for valine arylamidase, cysteine arylamidase, chymotrypsin, phosphoaminidase, acid phosphatase and glycyl-tryptophan arylamidase. Alkaline phosphatase and *N*-acetyl- β -glucosaminidase activities are weak or absent. No activity is observed for esterase [C-4], ester lipase [C-8], lipase [C-14], trypsin, α -glucosidase, β -glucosidase, α -mannosidase, β -mannosidase, α -fucosidase, α -galactosidase, β -galactosidase and β -glucuronidase. Voges-Proskauer

test is negative. Aesculin, gelatin and hippurate are not hydrolysed. Nitrate is not reduced to nitrite. Isolated from a juvenile elephant seal. The type strain, A/G13/99/2^T (= CCUG 42728^T = CIP 106764^T), was isolated from a nasal swab.

Acknowledgements

SAC Veterinary Science Division is supported by the Scottish Executive, Rural Affairs Department. We are grateful to Hans Trüper for help in deriving the species name.

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