Jeotgalicoccus pinnipedialis sp. nov., from a southern elephant seal (*Mirounga leonina*)

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A previously unknown Gram-positive, catalase-positive, facultatively anaerobic, non-spore-forming, coccus-shaped bacterium (A/G14/99/10^T), originating from the mouth of a female southern elephant seal, was subjected to a taxonomic analysis. Comparative 16S rRNA gene-sequencing showed that the organism formed a hitherto unknown subline within the catalase-positive, low-G + C, Gram-positive cocci, exhibiting a specific association with species of the genus *Jeotgalicoccus*. Sequence divergence values of approximately 7 %, together with phenotypic differences, showed the unknown bacterium to be distinct from the two described species of this genus, *Jeotgalicoccus halotolerans* and *Jeotgalicoccus psychrophilus*. Based on phenotypic and phylogenetic considerations, it is proposed that strain A/G14/99/10^T = CCUG 42722^T = CIP 107946^T from the mouth of a seal be classified as the type strain of a novel species of the genus *Jeotgalicoccus*, *Jeotgalicoccus pinnipedialis* sp. nov.

The genus *Jeotgalicoccus* was proposed by Yoon *et al.* (2003) to accommodate some Gram-positive, non-motile, catalase- and oxidase-positive, coccus-shaped organisms isolated from jeotgal, a traditional Korean fermented seafood. Two species of the genus, *Jeotgalicoccus halotolerans* and *Jeotgalicoccus psychrophilus*, are currently recognized. The genus *Jeotgalicoccus* forms a phylogenetically distinct line of descent that is close to, but separate from, members of the genus *Salinicoccus* and other catalase-positive, coccus-shaped genera (Yoon *et al.*, 2003). During the course of a study of taxonomically problematic organisms isolated from seals, we have characterized a *Jeotgalicoccus*-like organism from the oral cavity of a southern elephant seal. Based on the results of a polyphasic taxonomic study, it is clear that this coccus-shaped organism represents a novel species of the genus *Jeotgalicoccus*, for which we propose the name *Jeotgalicoccus pinnipedialis*.

Strain A/G14/99/10^T was isolated from a mouth swab taken from a female southern elephant seal as part of a British Antarctic Survey study in the South Orkneys, in 1993. The strain was grown aerobically at 37 °C on Columbia agar (Oxoid) supplemented with 5 % sheep blood. It was

characterized biochemically using the API STAPH, API ID32STAPH, API CORYNE and API ZYM systems according to the manufacturer's instructions (bioMérieux). For cellular fatty acid determination, the organism was grown on sheep blood agar at 37 °C for 3 days, and methyl esters were analysed using the MIDI microbial identification system. The G + C content of the DNA of strain A/G14/99/10^T was determined by HPLC according to Mesbah *et al.* (1989). Isoprenoid quinones were extracted as described by Collins *et al.* (1977) and analysed by HPLC as described by Groth *et al.* (1997). Polar lipids were extracted by the method of Minnikin *et al.* (1979) and analysed by two-dimensional TLC and by spraying with specific reagents (Collins & Jones, 1980). The 16S rRNA gene of the isolate was amplified by PCR and directly sequenced using a *Taq* dye-deoxy terminator cycle sequencing kit (Applied Biosystems) and an automated DNA sequencer (model 377, Applied Biosystems). The closest known relatives of the novel isolate were determined by performing database searches in the GenBank/EMBL data libraries. The determined sequence and those of its nearest phylogenetic relatives were aligned using the program CLUSTAL W (Thompson *et al.*, 1994). The resulting multiple sequence-alignment was corrected manually, and a distance matrix was calculated using the program DNADIST (using the Kimura 2-correction parameter) (Felsenstein, 1989). A

The GenBank EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Jeotgalicoccus pinnipedialis* A/G14/99/10^T is AJ251530.

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 (500 replications) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

Strain A/G14/99/10^T stained Gram-positive, and upon microscopic examination appeared as non-motile cocci (approximately 0.7–1 µm in diameter) arranged in a typical *Staphylococcus aureus* conformation (i.e. in 'bunches of grapes'), in pairs and in tetrads. Cells were non-acid fast and non-spore forming. The strain grew both aerobically and in an enriched-CO₂ environment, and was catalase- and oxidase-positive. It also grew in 2 and 6 % NaCl but not in 0 or 14 % NaCl. The organism failed to give any positive reactions with API STAPH. Using the API ID32STAPH system, weak activity for pyrrolidonyl arylamidase was detected; all other tests gave negative results with this system. Using the API ZYM system, activity was detected for acid phosphatase, phosphoamidase and ester lipase C8 (weak reaction); no other enzymes were detected with this system. Using the API CORYNE system, positive results were obtained for pyrrolidonyl arylamidase, pyrazinamidase and gelatin hydrolysis. The long-chain cellular fatty acids of the organism were found to be primarily of the iso- and anteiso-methyl branched-chain types. The major acids corresponded to anteiso-C_{15:0} (60 %) and iso-C_{15:0} (22.9 %), with other acids [namely anteiso-C_{13:0} (0.8 %), iso-C_{13:0} (1 %), C_{14:0} (1.5 %), iso-C_{14:0} (1.6 %), C_{16:0} (2.2 %), iso-C_{16:0} (1.6 %), iso-C_{17:0} (1.9 %), anteiso-C_{17:0} (4.5 %), iso-C_{18:0} (0.6 %) and iso-C_{19:0} (0.4 %)] present in only minor amounts. The major respiratory quinone of strain A/G14/99/10^T was MK-7 (89 %), with MK-8 (3 %) and MK-6 (5 %) present in minor amounts. The polar lipids of the strain consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and an unidentified phospholipid. No aminolipids or glycolipids were detected. The G + C content of the DNA of strain A/G14/99/10^T was 38.6 mol%. To ascertain the phylogenetic position of strain A/G14/99/10^T, its almost complete 16S rRNA gene sequence (1419 bp) was determined. Sequence database

searches revealed the strain to be most closely related to species of the genera *Jeotgalicoccus* and *Salinicoccus* (approximately 7 and 9 % sequence divergence, respectively), with species of other genera more distantly related (data not shown). A tree, constructed using the neighbour-joining method, depicting the phylogenetic relationships of strain A/G14/99/10^T, is shown in Fig. 1. The strain formed a distinct subline within the low-G + C, Gram-positive cocci, branching at the periphery of a cluster formed by *J. halotolerans* and *J. psychrophilus*. The association of strain A/G14/99/10^T with the genus *Jeotgalicoccus* was statistically highly significant (100 % bootstrap resampling value). Species of the genera *Macroccoccus* and *Salinicoccus* were the next nearest relatives of the novel strain, but they formed quite separate and robust clusters (Fig. 1).

From the comparative 16S rRNA gene sequence analysis, it is evident that the unidentified, catalase-positive, coccus-shaped organism represents a hitherto unknown taxon. Phylogenetically, strain A/G14/99/10^T displays a significant affinity with the genus *Jeotgalicoccus*. This association with *Jeotgalicoccus* is also consistent with phenotypic criteria, including chemical biomarkers. In particular, the long-chain cellular fatty acid profile of strain A/G14/99/10^T is very similar to that reported (Yoon *et al.*, 2003) for other *Jeotgalicoccus* species (i.e., primarily methyl branched-chain acids with anteiso-C_{15:0} predominating). Fatty acid composition is known to be dependent on growth conditions, and the observed minor quantitative differences between species possibly reflect the different media and culture conditions employed in the present study. The menaquinones and polar lipids of the unknown bacterium also closely resembled those of *J. halotolerans* and *J. psychrophilus* (Yoon *et al.*, 2003). By contrast, the absence of glycolipids and the synthesis of predominantly menaquinones with seven isoprene units serve to distinguish the seal bacterium from members of the genus *Salinicoccus*, which possess glycolipids and produce MK-6 as the major menaquinone (Ventosa *et al.*, 1990, 1992). The DNA G + C content of strain A/G14/99/10^T (38.6 mol%) is also significantly lower than that of *Salinicoccus* species (approximately

Fig. 1. Neighbour-joining tree showing the affiliation of *Jeotgalicoccus pinnipedialis* sp. nov. A/G14/99/10^T with members of the genus *Jeotgalicoccus*. The tree was based on an analysis of approximately 1350 bases. Statistically significant bootstrap values are shown at the nodes and are expressed as a percentage of 500 replications.

46–51 mol%) (Ventosa *et al.*, 1990, 1992), but is close to the value reported for *Jeotgalicoccus* species (42 mol%) (Yoon *et al.*, 2003). From 16S rRNA gene-sequence divergence considerations (7.5% from both *J. psychrophilus* and *J. halotolerans*, corresponding to 94 mismatches and 12 unmatched bases out of 1419), it is clear that strain A/G14/99/10^T merits classification as a distinct species of the genus *Jeotgalicoccus*, forming a relatively deep branch within the genus. Overall, phenotypically, the strain closely resembles *J. halotolerans* and *J. psychrophilus*. However, unlike *J. psychrophilus*, strain A/G14/99/10^T can grow at 37 and 42 °C. It further differs from *J. psychrophilus* by failing to grow in 14% NaCl or at 4 °C. Growth in NaCl also serves to distinguish strain A/G14/99/10^T from *J. halotolerans*, since unlike *J. halotolerans*, the seal isolate does not grow in the absence of NaCl or in the presence of 20% NaCl (Yoon *et al.*, 2003). Therefore, on the basis of phenotypic and phylogenetic evidence, we consider strain A/G14/99/10^T to merit classification as a novel species of the genus *Jeotgalicoccus*, for which we propose the name *Jeotgalicoccus pinnipedialis* sp. nov. Tests that are useful in distinguishing *J. pinnipedialis* from *J. psychrophilus* and *J. halotolerans* are shown in Table 1.

Description of *Jeotgalicoccus pinnipedialis* sp. nov.

Jeotgalicoccus pinnipedialis (pin.ni.ped.i.a'lis. N.L. masc. adj. *pinnipedialis* pertaining to pinnipeds).

Cells stain Gram-positive and are coccus-shaped, appearing in 'bunches of grapes', in pairs or in tetrads. Cells are non-acid fast and non-motile. Colonies on blood agar are non-haemolytic, buff or fawn, round, convex and approximately 2 mm in diameter after 24 h aerobic incubation. Colonies have a similar appearance on nutrient agar. Growth is not enhanced by increased concentrations of CO₂. Grows at 25 and 42 °C, but not at 4 °C. Growth occurs

in 2 and 6% NaCl but not in 0 or 14% NaCl. Catalase- and oxidase-positive. Using API systems, acid is not produced from arabinose, cellobiose, glucose, glycogen, fructose, lactose, mannose, mannitol, maltose, melibiose, α -methyl D-glucoside (methyl α -D-glucopyranoside), raffinose, ribose, sucrose, trehalose, turanose, xylitol or D-xylose. Gelatin is hydrolysed, but aesculin and hippurate are not. Activity for acid phosphatase, phosphoamidase, pyrazin-aminidase and pyrrolidonyl arylamidase is detected. Activity for ester lipase C8 is either weak or absent. No activity is observed for alkaline phosphatase, arginine dihydrolase, arginine arylamidase, chymotrypsin, cystine arylamidase, esterase C4, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, leucine arylamidase, lipase C14, α -mannosidase, ornithine decarboxylase, N-acetylglucosaminidase, trypsin, valine arylamidase or urease. Acetoin is not produced. Nitrate is not reduced to nitrite. The long-chain cellular fatty acids are primarily of the anteiso- and iso-methyl branched types, with anteiso-C_{15:0} and iso-C_{15:0} predominating. Unsaturated menaquinones with 7 isoprene units (MK-7) are the predominant respiratory lipoquinones. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidyl-inositol and an unidentified phospholipid.

The type strain is A/G14/99/10^T (=CCUG 42722^T = CIP107946^T). The G+C content of its DNA is 38.6 mol%. Isolated from a mouth swab taken from a southern elephant seal. Habitat is not known.

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Table 1. Differential phenotypic characteristics of *J. pinnipedialis*, *J. halotolerans* and *J. psychrophilus*

Characteristic	<i>J. pinnipedialis</i>	<i>J. halotolerans</i> *	<i>J. psychrophilus</i> *
Growth at:			
4 °C	–	+	+
37 °C	+	+	–
42 °C	+	+	–
Growth in NaCl:			
0 %	–	+	–
14 %	–	+	+
20 %	–	+	–
Acid production from:			
Arabinose	–	+	–
D-Mannitol	–	+	–

*Data for reference species taken from Yoon *et al.* (2003).

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