NOTE

***Vagococcus fessus* sp. nov., isolated from a seal and a harbour porpoise**

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A polyphasic taxonomic study was performed on two strains of an unknown Gram-positive, catalase-negative, coccus-shaped bacterium isolated from a dead seal and a harbour porpoise. Comparative 16S rRNA gene sequencing demonstrated that the unknown bacterium represents a new subspecies within the genus *Vagococcus* close to, but distinct from, *Vagococcus fluvialis*, *Vagococcus lutrae* and *Vagococcus salmoninarum*. The unknown bacterium was readily distinguished from the three currently recognized *Vagococcus* species 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 a new species, *Vagococcus fessus*. The type strain of *Vagococcus fessus* is CCUG 41755^T.

Keywords: *Vagococcus fessus* sp. nov., taxonomy, phylogeny, 16S rRNA

The genus *Vagococcus* was proposed (Collins *et al.*, 1989) to accommodate a phylogenetically distinct group of Gram-positive, motile, coccus-shaped organisms which resembled lactococci in reacting with Lancefield group N antisera. The genus was originally monospecific, containing the species *Vagococcus fluvialis*, isolated from chicken faeces and river water (Hashimoto *et al.*, 1974). *V. fluvialis* has since been recovered from diverse sources including human clinical specimens, viz. blood, peritoneal fluid and wounds (Teixeira *et al.*, 1997) and various domestic animals, viz. chickens, pigs, cattle, horses and cats (Pot *et al.*, 1994a). Two further species, *Vagococcus salmoninarum* (Wallbanks *et al.*, 1990) and *Vagococcus lutrae* (Lawson *et al.*, 1999), have subsequently been assigned to the genus. *V. salmoninarum* has been recovered from diseased fish (e.g. Atlantic salmon, rainbow trout and brown trout with peritonitis; Schmidtke & Carson, 1994) whereas the only known strain of *V. lutrae* originated from the common otter (*Lutra lutra*) (Lawson *et al.*, 1999). In this article, the results of a polyphasic taxonomic study on two biochemically unreactive *Vagococcus*-like organisms isolated following post-mortem examinations of a seal and a harbour porpoise are reported. On the basis of the presented

findings, it is considered that the unknown coccus represent a fourth species of the genus *Vagococcus*, for which the name *Vagococcus fessus* sp. nov., is proposed.

Two unknown coccus-shaped organisms designated M2661/98/1^T (=CCUG 41755^T) and M520/99/2 (=CCUG 42419) were isolated from a dead seal and harbour porpoise, respectively. The isolate from the seal carcass was recovered in pure growth from liver and kidney whereas the organism from the dead porpoise was obtained in heavy growth from multiple organs (peritoneum, spleen, kidney, liver, lung, brain, placenta and small intestine). The only other isolates from the latter animal were scant growth of a host-adapted *Salmonella* from the lung and a *Proteus* from the brain. Both isolates stained Gram-positive and were catalase-negative. The unidentified organisms were cultured on Columbia agar supplemented with 5% defibrinated horse blood (Oxoid) at 37 °C, in air plus 5% CO₂. The strains were biochemically characterized by using the API Rapid ID32S and API ZYM systems according to the manufacturer's instructions (API bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994b). For densitometric analysis, normalization and interpretation of protein patterns, the GELCOMP GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used. Similarity between pairs of traces was carried out using the UPGMA clustering method

Abbreviation: RDP, Ribosomal Database Project.

The GenBank accession number for the 16S rRNA gene of CCUG 41755^T is AJ243326.

Fig. 1. Similarity dendrogram based on whole-cell protein pattern of *Vagococcus fessus* sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

and expressed by the Pearson product-moment correlation coefficient converted for convenience to a percentage value (Pot *et al.*, 1994b). 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 the GenBank or Ribosomal Database Project (RDP) databases and aligned with the newly determined sequence using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the programs PRETTY and DNADIST (using the Kimura-two 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) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The unknown organisms recovered from the seal and porpoise consisted of Gram-positive, coccus-shaped cells which occurred singly, in pairs or in chains, and were elongated in the direction of the chain. The isolates were catalase-negative, facultatively anaerobic and produced acid but not gas from glucose. Using the API Rapid ID32S system, the organisms were relatively unreactive, producing positive reactions for pyroglutamic acid arylamidase. Variable results were obtained for β -galactosidase, β -glucosidase and glycyl tryptophan arylamidase. The production of acid from cyclodextrin was also variable. All other tests were negative. Using the API ZYM system, the unknown

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Vagococcus fessus* sp. nov. and some other low-G+C-content Gram-positive bacteria. The tree, constructed using the neighbour-joining method, was based on a comparison of approx. 1320 nt. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points; only values above 90% are shown. Bar, sequence divergence range of 1%.

cocci showed activity for esterase C4 (weak), ester lipase C8 (weak), phosphoamidase (weak), chymotrypsin and leucine arylamidase. Variable results were obtained for alkaline phosphatase and cystine arylamidase. No other enzymic activities were detected. On the basis of their general unreactivity, the unidentified cocci somewhat resembled gemellae and some *Facklamia* species. However, their biochemical reactions did not correspond closely to any described species. PAGE analysis of whole-cell proteins showed that the unknown organisms were quite distinct from gemellae and *Facklamia* species. On the basis of PAGE analysis, the unknown organisms clustered together and showed highest similarity with *Alloicoccus otitis* and *Tetragenococcus halophilus*, although this association was not particularly close. A dendrogram illustrating the results of PAGE analysis is shown in Fig. 1 and shows that the unknown isolates are phenotypically separate from all other Gram-positive, catalase-negative reference species examined. Comparative 16S rRNA gene sequencing was performed in order to determine the phylogenetic affinities of the two unknown cocci. The determined sequences

(>1470 nt) of the two unidentified organisms were found to be identical (100% sequence similarity) to each other. Sequence searches of GenBank and RDP databases revealed that the newly determined sequences were most highly related to vagococci (95.2–95.6%). A tree constructed by neighbour-joining depicting the phylogenetic relationships of the unknown bacterium is shown in Fig. 2. Treeing analysis confirmed the close association of the organism with the genus *Vagococcus*. The clustering together of the unknown bacterium and vagococci was found in 91% of 500 tree replications.

The two unidentified cocci isolated from seal and porpoise were found to be biochemically related and formed a distinct group on PAGE protein analysis. The finding of identical 16S rRNA gene sequences in the two isolates was consistent with their phenotypic similarities and is indicative of the same species. Phylogenetically, the unknown species forms a robust cluster with vagococci branching near to the base of the genus. This association was statistically significant, although sequence divergence values of approximately

4.4–4.8% between the unknown bacterium and the three currently recognized *Vagococcus* species clearly demonstrate that the coccus-shaped organism represents a genetically distinct species. Biochemically, the unknown species most closely resembles the fish pathogen *V. salmoninarum*. However, the new species can be distinguished from *V. salmoninarum* in that it does not produce acid from trehalose and it is valine arylamidase-negative. In contrast, *V. salmoninarum* is positive for both of these reactions. The unknown coccus could be readily distinguished from *V. fluvialis* and *V. lutrae* by its general unreactivity towards sugars. For example, the new bacterium does not produce acid from maltose, ribose, sorbitol, sucrose or trehalose whereas *V. fluvialis* and *V. lutrae* ferment these substrates. Thus, based on the phylogenetic and phenotypic distinctiveness of the unknown bacterium, it is proposed that the unknown coccus be classified as a new species of the genus *Vagococcus*, *Vagococcus fessus*.

Description of *Vagococcus fessus* sp. nov.

Vagococcus fessus (fes.sus. L. adj. *fessus* weary, pertaining to the general biochemical unreactivity of the organism).

Cells are Gram-positive cocci that occur as single cells, in pairs or in chains. Cells are elongated in the direction of the chain. α -Haemolytic on blood agar. Facultatively anaerobic and catalase-negative. Acid but no gas is produced from glucose metabolism. Using API systems, acid may or may not be produced from cyclodextrin. Acid is not produced from D-arabitol, L-arabinose, glycogen, lactose, mannitol, melibiose, methyl β -D-glucopyranoside, melezitose, pullulan, D-raffinose, D-ribose, sorbitol, sucrose, tagatose, trehalose or D-xylose. Activities for chymotrypsin, esterase C4 (weak), ester lipase C8 (weak), phosphoamidase (weak), pyroglutamic acid arylamidase and leucine arylamidase are detected. Activity may or may not be detected for alkaline phosphatase, cystine arylamidase, β -galactosidase, β -glucosidase and glycyl tryptophan arylamidase. No activity for alanyl phenylalanine proline arylamidase, arginine dihydrolase, N-acetyl- β -glucosaminidase, α -fucosidase, α -galactosidase, β -galactosidase, α -galacturonidase, α -glucosidase, β -glucuronidase, α -mannosidase, β -mannosidase, lipase C14, trypsin, urease or valine arylamidase is detected. Hippurate is not hydrolysed. Nitrate is not reduced. Voges-Proskauer test is negative. The DNA G + C content is 40.5 mol%. The type strain is CCUG 41755^T, which was isolated from a

dead seal. A second strain was recovered from multiple organs from a dead harbour porpoise. Pathological significance is not known.

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