NOTE***Vagococcus lutrae* sp. nov., isolated from the common otter (*Lutra lutra*)**

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Phenotypic and phylogenetic studies were performed on an unknown Gram-positive catalase-negative coccus isolated from a common otter (*Lutra lutra*). Comparative 16S rRNA gene sequencing demonstrated that the unknown bacterium represents a new subline within the genus *Vagococcus*, close to, but distinct from, *Vagococcus fluvialis* and *Vagococcus salmoninarum*. The unknown bacterium was readily distinguished from the two 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 lutrae*, the type strain of which is CCUG 39187^T.

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

The genus *Vagococcus* was described by Collins *et al.* (1989) to accommodate some motile Lancefield group N Gram-positive, catalase-negative cocci, which were phylogenetically distinct from lactococci. Initially the genus consisted of a single species, *Vagococcus fluvialis*, which was isolated from chicken faeces and river water and first described by Hashimoto *et al.* (1974). *V. fluvialis* has subsequently been reported from a variety of sources including human clinical specimens, viz. blood, peritoneal fluid and wounds (Teixeira *et al.*, 1997) and domestic animals, viz. chickens, pigs, cattle, horses and cats (Pot *et al.*, 1994a). A second species of the genus, *Vagococcus salmoninarum*, was described by Wallbanks *et al.* (1990) for two strains isolated from diseased rainbow trout in the USA. *V. salmoninarum* has subsequently been recovered from Atlantic salmon, rainbow trout and brown trout with peritonitis (Schmidtke & Carson, 1994). In the course of a study of Gram-positive catalase-negative cocci associated with aquatic mammals, we have used 16S rRNA gene sequencing to phylogenetically characterize a hitherto-unknown *Vagococcus*-like bacterium from a common otter. Based on the results of a polyphasic taxonomic study, we consider the unknown coccus represents a third species of the genus *Vagococcus*, for which the name *Vagococcus lutrae* sp. nov., is proposed.

An unknown coccus was isolated following a post-mortem examination of a common otter following a road traffic accident on the Isle of Mull, UK. The coccus was recovered from the blood, liver, lungs and spleen specimens from the otter as the dominant organism co-isolated with an unidentified oxidase-positive, non-fermentative, non-motile, Gram-negative rod-shaped bacterium. All of the coccoid isolates recovered from these multiple sites of the otter were found to possess identical cellular and colonial morphologies and biochemical profiles. The post-mortem revealed the animal to be in good bodily condition prior to the road traffic accident and therefore it was not possible to draw conclusions regarding the possible, if any, clinical significance of the unknown isolate. Isolate M1134/97/1^T was deposited in the Culture Collection of the University of Göteborg (CCUG) Sweden, as number CCUG 39187^T. The unidentified organism was cultured on Columbia agar supplemented with 5% defibrinated horse blood (Oxoid Unipath) at 37 °C, in air plus 5% CO₂. The strain was 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). The GELCOMP GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used for densitometric analysis, normalization and interpretation of protein patterns. Similarity between pairs of traces was carried out using the unweighted pair group method with

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 39187^T is Y17152.

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

averages (UPGMA) clustering method 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 isolate were amplified by PCR and directly sequenced using a *Taq* Dye-Deoxy terminator cycle sequencing kit and a model 373A automatic DNA sequencer (both from Applied Biosystems). 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 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 bacterium from the common otter consisted of Gram-positive coccus-shaped cells which occurred singly, in pairs or short chains, and which were elongated in the direction of the chain. The isolate was catalase-negative, facultatively anaerobic and motile. It produced small (0.1–0.2 mm diameter) smooth colonies on Columbia agar supplemented with 5% defibrinated horse blood at 37 °C. The isolate produced acid from glucose and some other carbohydrates, and displayed acid phosphatase, *N*-acetylglucosaminidase, chymotrypsin, ester lipase C-8, α -

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Vagococcus lutrae* 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 approximately 1320 nucleotides. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points. The bar represents a sequence divergence range of 1%.

galactosidase, α -glucosidase, β -glucosidase, glycyl-tryptophan arylamidase, leucine arylamidase, β -mannosidase and pyroglutamic acid arylamidase activity. The unknown bacterium hydrolysed aesculin, produced acetoin, but did not reduce nitrate. The detailed biochemical characteristics of the unknown coccus are presented in the species description of this organism. PAGE analysis of whole-cell proteins showed the unknown organism to be separate from all other Gram-positive catalase-negative reference species examined, including *Vagococcus* spp., *Abiotrophia* spp., *Aerococcus* spp., *Carnobacterium* spp., *Facklamia hominis* and *Globicatella sanguinis* (Fig. 1). On the basis of PAGE analysis the unknown coccus formed a loose association with *Aerococcus urinae*. By contrast *V. fluvialis* and *V. salmoninarum* were far-removed from the unknown bacterium. To assess the genealogical affinity between the unidentified organism and other Gram-positive catalase-negative taxa, comparative 16S rRNA gene sequence analyses were performed. The almost complete gene sequence (>1470 nucleotides) of the unknown coccus was determined. Sequence searches of GenBank and RDP databases revealed the unknown bacterium was phylogenetically most closely associated with the Lactic acid group of bacteria. A tree constructed by neighbour-joining depicting the phylogenetic relationships of the unknown coccus is shown in Fig. 2. It was evident

Table 1. Characteristics that differentiate *V. lutrae* sp. nov. from *V. fluvialis* and *V. salmoninarum*

Data derived from this study; *n*, number of strains studied; results for *V. salmoninarum* (*n* = 2) were all negative.

Test	<i>V. lutrae</i> (<i>n</i> = 1)	<i>V. fluvialis</i> (<i>n</i> = 6)
Acid from:		
Cyclodextrin	+	+
Mannitol	—	+
Sorbitol	+	+
Sucrose	+	+
D-Ribose	+	+
Production of:		
α -Galactosidase	+	—
β -Galactosidase	+	—

from the treeing analysis that the nearest relatives of the unidentified coccus were *V. fluvialis* and *V. salmoninarum*. The clustering together of these organisms occurred in 100% of 500 tree replications. Despite the statistically significant association between the unknown coccus and the *Vagococcus* species, pairwise comparisons showed the 16S rRNA of the unidentified organism possessed 57 and 76 base

differences (based on 1470 positions) with *V. fluvialis* and *V. salmoninarum*, respectively (corresponding to >3.8% 16S rRNA sequence divergence). This degree of sequence divergence is comparable to that shown between *V. fluvialis* and *V. salmoninarum* (4.4%).

The unknown coccus from the common otter formed a phylogenetically robust cluster with *V. fluvialis* and *V. salmoninarum*. Sequence divergence values of approximately 3.8 and 4.8% between the unknown coccus and these two species respectively, however demonstrates this relationship is that of phylogenetically closely related but nevertheless quite separate, species. Thus, based on the phylogenetic findings we propose the unknown coccus to be classified as a new species of the genus *Vagococcus*, *Vagococcus lutrae* sp. nov. Examples of characteristics which serve to distinguish the new coccus from *V. fluvialis* and *V. salmoninarum* are outlined in Table 1.

Description of *Vagococcus lutrae* sp. nov.

Vagococcus lutrae (luo'trae. M.L. fem. gen. n. *lutrae* of or pertaining to the common otter, *Lutra lutra*, the mammal from which the bacterium was isolated).

Cells are Gram-positive cocci occurring as single cells, in pairs or in short chains elongated in the direction of the chain. Cells are non-spore-forming and motile. Small, smooth colonies up to 0.2 mm in diameter are formed on blood agar at 37 °C. Facultatively anaerobic and catalase-negative. Acid is produced from cyclodextrin, glucose, maltose, methyl- β -D-glucopyranoside, D-ribose, sorbitol, sucrose and trehalose. Acid was not produced from D-arabitol, L-arabinose, glycogen, mannitol, melibiose, melezitose, pullulan, D-raffinose, tagatose or D-xylose. Acid phosphatase, *N*-acetylglucosaminidase, chymotrypsin, ester lipase C-8, α -galactosidase, α -glucosidase, β -glucosidase, glycyl-tryptophan arylamidase, leucine arylamidase, β -mannosidase and pyroglutamic acid arylamidase activity is detected. No activity for alanine-phenylalanine-proline arylamidase, arginine dihydrolase, α -fucosidase, β -glucuronidase, α -mannosidase, lipase C-

14, trypsin, urease or valine arylamidase is detected. Activity of alkaline phosphatase, β -galactosidase and β -galacturonidase is either weak or negative. Aesculin is hydrolysed, but hippurate and gelatin are not. Nitrate is not reduced. Voges-Proskauer test is negative. Isolated from the common otter. Habitat not known. The type strain is CCUG 39187^T.

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