Vagococcus elongatus sp. nov., isolated from a swine-manure storage pit

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A polyphasic taxonomic study was performed on an uncharacterized Gram-positive, catalase-negative, elongated coccus-shaped bacterium isolated from a swine-manure storage pit. The bacterium, designated strain PPC9^T, was facultatively anaerobic and had a DNA G + C content of 44.5 mol%. Comparative 16S rRNA gene sequencing indicated that the bacterium represented a novel subline within the genus *Vagococcus*, close to but distinct from *Vagococcus lutrae*. Strain PPC9^T was readily distinguished from the five recognized species of the genus *Vagococcus* by using biochemical tests and molecular genetic analysis. Based on phylogenetic and phenotypic evidence strain PPC9^T is considered to represent a novel species of the genus *Vagococcus*, for which the name *Vagococcus elongatus* sp. nov. is proposed. The type strain is PPC9^T (=CCUG 51432^T =NRRL B-41357^T).

The genus *Vagococcus* was proposed by Collins *et al.* (1989) to accommodate a phylogenetically distinct group of Gram-positive, motile, coccus-shaped organisms that resembled lactococci in reacting with Lancefield group N antisera. The genus was originally monospecific, comprising 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 (blood, peritoneal fluid and wounds; Teixeira *et al.*, 1997) and various domestic animals (chickens, pigs, cattle, horses and cats; Pot *et al.*, 1994). Four further species have subsequently been assigned to the genus. *Vagococcus salmoninarum* has been recovered from diseased fish (e.g. Atlantic salmon, rainbow trout and brown trout with peritonitis; Schmidtke & Carson, 1994), *Vagococcus fessus* (Hoyle *et al.*, 2000) has been isolated from a seal and a harbour porpoise, *Vagococcus lutrae* originated from the common otter (Lawson *et al.*, 1999) while *Vagococcus carniphilus* was isolated from ground beef (Shewmaker *et al.*, 2004). Here we report the results of a polyphasic taxonomic study on a strain of a *Vagococcus*-like organism isolated from a swine-manure storage pit. On the basis of the findings presented we consider that this bacterium represents a novel species of the genus *Vagococcus*.

An unknown, elongated coccus-shaped organism, designated strain PPC9^T, was isolated from a swine-manure storage pit. The isolate stained Gram-positive and was catalase-negative. The unidentified organism was cultured on basic media containing macrominerals, microminerals, buffers, reducing agents and other components as in the routine growth medium described by Hespell *et al.* (1987), with 0.2 % carbohydrate added as a carbon source. All media used in these experiments were prepared anaerobically by using the method of Hungate as modified by Bryant (1972). Strain PPC9^T was characterized biochemically by using the API Rapid ID32S and API ZYM systems at 37 °C for 4 h aerobically, according to the manufacturer's instructions (API bioMérieux). Determination of the DNA G + C content was carried out by thermal denaturation of chromosomal DNA by using a Beckman model DU 640 spectrophotometer equipped with a high-performance temperature controller and *T_m* analysis software (Johnson, 1994). 16S rRNA gene fragments were generated by PCR with universal primers pA (positions 8–28 according to the *Escherichia coli* numbering system) and pH* (1542–1522). The amplified product was purified by using a QIAquick PCR purification kit and sequenced directly by using primers to conserved regions of the 16S rRNA gene. Sequencing was performed by using a PRISM Taq Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PPC9^T is AF445297.

the new isolate were determined by performing database searches with the program FASTA (Pearson & Lipman, 1985). These sequences and those of strains of related recognized species were retrieved from GenBank and aligned with the newly determined sequence by using the program SEQtools (Rasmussen, 2002). The resulting multiple sequence alignment was corrected manually with the program GeneDoc (Nicholas *et al.*, 1997) and a phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987) with the programs SEQtools and TreeView (Page, 1996). The stability of the groupings was estimated by bootstrap analysis (1000 replications) with the same programs.

Cells of strain PPC9^T occurred in short chains and were elongated in the direction of the chain. This elongation was so pronounced that some cells gave the appearance of short, fat rods. The new isolate was facultatively anaerobic and produced lactic acid but not gas from glucose. Cells appeared to be non-motile based on a 0.4 % agar tube test (Bailey & Scott, 1974, pp. 376–377) and when observed microscopically. The organism grew optimally at 37 °C. When grown for 3 days under CO₂ on trypticase soy agar supplemented with 5 % defibrinated horse blood, it produced colonies that were grey, convex and approximately 0.5–2 mm in diameter. Growth under strictly anaerobic conditions was slower and incubation continued for 5–7 days. No haemolysis was observed. With the API Rapid ID32S system the organism was relatively inactive, producing positive reactions only for β -glucosidase (weak), α -galactosidase and glycyl tryptophan arylamidase. All other tests were negative. With the API ZYM system the unknown organism showed weak activity for alkaline phosphatase, esterase C4 and esterase lipase C8. No other enzymic activities were detected. The DNA G + C content of strain PPC9^T was 44.5 mol%.

Comparative 16S rRNA gene sequencing was performed in order to determine the phylogenetic affinities of the unknown bacterium. Searches of GenBank revealed that

the 16S rRNA gene sequence of strain PPC9^T was phylogenetically most closely related to those of the lactic acid group of bacteria and in particular was most highly related to those of vagococci (94.0–96.7 % sequence similarity). A neighbour-joining tree depicting the phylogenetic relationships of strain PPC9^T is shown in Fig. 1. The treeing analysis confirmed the close association of the organism with members of the genus *Vagococcus*. Strain PPC9^T formed a distinct subline based on this comparative 16S rRNA gene sequence analysis sharing a branching node with *V. lutrae* CCUG 39187^T (96.7 % similarity). Phylogenetically, strain PPC9^T formed a robust cluster with the vagococci; the association was statistically significant, although sequence divergence values of approximately 4.1–6 % between the unknown bacterium and the five recognized *Vagococcus* species clearly demonstrate that strain PPC9^T represents a genetically distinct species. There is no precise correlation between level of 16S rRNA gene sequence divergence and species delineation, but it is generally recognized that divergence values of 3 % or more are significant (Stackebrandt & Goebel, 1994). Support for the distinctiveness of the unknown manure bacterium was also evident from phenotypic analyses. Biochemically, the unknown species most closely resembled *V. fessus* isolated from a seal and harbour porpoise based on their shared generally unreactive biochemical properties. However, strain PPC9^T could be distinguished from *V. fessus* in not producing the enzymes α -chymotrypsin or pyroglutamic acid arylamidase and by its production of β -glucosidase (weak) and α -galactosidase. In addition to 16S rRNA gene sequence analysis data, strain PPC9^T could be readily distinguished from the other recognized *Vagococcus* species based on the traits given in Table 1. In particular, the production of API Rapid ID32S numerical profile 22000400000 served to distinguish the novel bacterium from all recognized *Vagococcus* species. Thus, based on the phylogenetic and phenotypic distinctiveness of strain PPC9^T we consider that it should be classified as representing a novel species of the genus *Vagococcus*, for which the name

Fig. 1. Unrooted tree showing the phylogenetic relationships of strain PPC9^T and some other Gram-positive bacteria with low DNA G+C content. The tree was constructed by using the neighbour-joining method based on a comparison of 16S rRNA gene sequences of approximately 1320 nt. Bootstrap values, expressed as a percentage of 1000 replications, are given at branching points; only values ≥ 90 % are shown. Bar, 1 % sequence divergence.

Table 1. Characteristics that differentiate strain PPC9^T from other *Vagococcus* species

Strains: 1, PPC9^T; 2, *V. carniphilus* CCUG 32704^T; 3, *V. fessus* CCUG 41755^T; 4, *V. fluvialis* CCUG 32704^T; 5, *V. lutrae* CCUG 39187^T; 6, *V. salmoninarum* CCUG 33394^T. Data for reference strains were taken from the CCUG database (<http://www.ccug.se>).
–, Negative; +, positive; +^w, weak reaction.

Characteristic	1	2	3	4	5	6
Acid from:						
β-D-Glucopyranoside	–	+	–	–	+	–
Cyclodextrin	–	+	–	+	+	–
Mannose	–	–	–	+	–	–
Ribose	–	+	–	+	+	–
Sorbitol	–	–	–	+ ^w	+	–
Trehalose	–	+	–	+	+	+
Production of:						
N-Acetyl-β-glucosaminidase	–	–	–	–	+	–
α-Chymotrypsin	–	–	+	+ ^w	+	+
β-Glucosidase	+ ^w	–	–	+	+	+
α-Galactosidase	+	–	–	–	+	–
β-Mannosidase	–	–	–	+ ^w	+	–
Pyroglutamic acid arylamidase	–	+	+	+ ^w	+	+

Vagococcus elongatus sp. nov. is proposed. Although only a single strain of *V. elongatus* is currently known, we consider that the formal description of this species, together with 16S rRNA gene sequence data and biochemical criteria to aid its identification, will facilitate its recognition in routine laboratory investigations, thereby permitting the recovery of additional strains.

Description of *Vagococcus elongatus* sp. nov.

Vagococcus elongatus (e.lon.ga'tus. L. masc. part. adj. *elongatus* elongated, pertaining to the pronounced elongation of the cells).

Cells are Gram-positive cocci occurring singly or in short chains, with cells elongated in the direction of the chain, and some giving the appearance of short, fat rods. Facultatively anaerobic and catalase-negative. Non-haemolytic and non-motile. Lactic acid but no gas is produced from glucose metabolism. No growth in broth containing 6.5 % NaCl. No growth at 10 or 45 °C. Using the API ID32S system, α-galactosidase, β-glucosidase (weak reaction) and glycyl tryptophan arylamidase are detected. Acid is not produced from N-acetyl-β-glucosaminidase, alkaline phosphatase, alanyl phenylalanine proline arylamidase, arginine dihydrolase, D-arabitol, L-arabinose, cyclodextrin, β-glucuronidase, β-galactosidase, glycogen, lactose, maltose, mannitol, α-mannosidase, melibiose, methyl β-D-glucopyranoside, melezitose, pyroglutamic acid arylamidase, pullulan, D-raffinose, D-ribose, sorbitol, sucrose, tagatose, trehalose or urease. Using the API ZYM system, weak reactions are

obtained for alkaline phosphatase, esterase C4 and esterase lipase C8. No activity is detected for acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), leucine arylamidase, α-mannosidase, α-fucosidase, trypsin or valine arylamidase. Nitrate is not reduced. Voges-Proskauer test is negative. Aesculin is hydrolysed but hippurate and gelatin are not. The DNA G + C content is 44.5 mol%.

The type strain, PPC9^T (=CCUG 51432^T=NRRL B-41357^T), was isolated from a swine-manure storage pit. Habitat unknown.

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