Vagococcus penaei sp. nov., isolated from spoilage microbiota of cooked shrimp (*Penaeus vannamei*)

Emmanuel Jaffrès,^{1,2} Hervé Prévost,¹ Albert Rossero,¹
Jean-Jacques Joffraud² and Xavier Dousset¹

Correspondence

Xavier Dousset

dousset@enitaa-nantes.fr

¹UMR INRA 1014 SECALIM ENITAA, rue de la Géraudière, BP 82225, 44322 Nantes Cedex 3, France

²Ifremer, Laboratoire Science et Technologie de la Biomasse Marine, BP 21105, 44311 Nantes Cedex 3, France

A polyphasic taxonomic study, using phenotypic, phylogenetic and genotypic characterization, was performed on five Gram-stain-positive, catalase-negative, coccus-shaped *Vagococcus*-like bacteria isolated from the spoilage microbiota of cooked shrimp. Comparative 16S rRNA gene sequence analysis indicated that the isolates belonged to the genus *Vagococcus*. The five isolates shared 100% 16S rRNA gene sequence similarity, and representative strain CD276^T formed a branch that was distinct from the type strains of the six recognized species of the genus *Vagococcus* (*Vagococcus fluvialis* CCUG 32704^T, *V. salmoninarum* NCFB 2777^T, *V. lutrae* CCUG 39187^T, *V. fessus* M2661/98/1^T, *V. carniphilus* ATCC BAA-340^T and *V. elongatus* PPC9^T). The taxonomic position of strain CD276^T was clarified using DNA–DNA hybridization, pulsed-field gel electrophoresis of whole-genome DNA, G + C content determination, cell-wall peptidoglycan typing, fatty acid analysis and biochemical characterization. On the basis of this evidence, a novel species, *Vagococcus penaei* sp. nov., is proposed. The type strain is CD276^T (=LMG 24833^T =CIP 109914^T).

The bacterial genus *Vagococcus* was proposed by Collins *et al.* (1989) in order to improve the taxonomy of Gram-positive, catalase-negative, motile, coccus-shaped bacteria that are close to the lactococci in terms of reacting with Lancefield group N antisera but are phylogenetically distant. Phylogenetic studies have shown that the closest relatives of the genus *Vagococcus* are the genera *Enterococcus* and *Carnobacterium* (Wallbanks *et al.*, 1990). To date, the genus *Vagococcus* consists of six species. The first described was *Vagococcus fluvialis*, which was isolated from chicken faeces and seawater (Hashimoto *et al.*, 1974), various internal organs of domestic animals such as pigs, cattle, cats and horses (Pot *et al.*, 1994), human clinical samples such as blood, peritoneal fluid and wounds (Teixeira *et al.*, 1997) and, more recently, from a root-filled tooth with periradicular lesions (Al-Ahmad *et al.*, 2008). *Vagococcus salmoninarum* was recognized by Wallbanks *et al.* (1990) and has been isolated from diseased fish such as Atlantic salmon, rainbow and brown trout (Michel *et al.*, 2007; Ruiz-Zarzuela *et al.*, 2005; Schmidtke & Carson, 1994; Wallbanks *et al.*, 1990). Two further species were found subsequently in marine mammals and allocated to the genus: *Vagococcus lutrae* (Lawson *et al.*,

1999), isolated from the common otter (*Lutra lutra*), and *Vagococcus fessus* (Hoyle *et al.*, 2000), from a seal and a harbour porpoise. In the last few years, two more species have been isolated: *Vagococcus carniphilus* from ground beef (Shewmaker *et al.*, 2004) and *Vagococcus elongatus* from a swine-manure storage pit (Lawson *et al.*, 2007). Recently, we isolated five *Vagococcus*-like bacteria from the microbiota of spoiled cooked shrimp (Jaffrès *et al.*, 2009). The present work describes the polyphasic taxonomic study of a representative strain, CD276^T.

The five isolates, designated CD276^T, CD279, CD285, CD310 and CD380, were collected from a batch of cooked, peeled, brined and drained shrimp (*Penaeus vannamei* harvested in the Pacific Ocean) that had been packaged in a modified atmosphere and were considered as spoiled by a trained panel experienced in the sensory evaluation of seafood. Details of the sensory and microbiological monitoring, strain collection and identification can be found in our previous study (Jaffrès *et al.*, 2009).

The phylogenetic position of the five isolates was determined by complete 16S rRNA gene sequence analysis. Genomic DNA extraction and purification were carried out using the DNeasy Blood and Tissue kit (Qiagen). Amplification, purification and sequencing of the 16S rRNA gene were performed as described by Valcheva *et al.* (2006). Sequence analysis using the BioEdit sequence

Abbreviation: PFGE, pulsed-field gel electrophoresis.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CD276^T is FJ360897.

alignment software (Hall, 1999) revealed that the five isolates shared 100% 16S rRNA gene sequence similarity. The 16S rRNA gene sequence of strain CD276^T and those of strains of the most closely related genera, *Vagococcus* and *Enterococcus*, from GenBank and the Ribosomal Database Project (<http://rdp.cme.msu.edu>; Cole *et al.*, 2003) were aligned using CLUSTAL in MEGA version 4.0 and a neighbour-joining phylogenetic tree was created (Tamura *et al.*, 2007) with Kimura's two-parameter model (Gascuel, 1997; Kimura, 1980) on 1425 gap-free sites (Fig. 1). Sequences from the genera *Vagococcus* and *Enterococcus* clustered into two distinct monophyletic groups, and the close association of strain CD276^T with members of the genus *Vagococcus* was confirmed by a bootstrap value of 99%. After realigning the distance matrix with CLUSTAL W, strain CD276^T formed a distinct branch with the four other isolates that shared a node with *V. carniphilus* ATCC BAA-640^T and *V. fluvialis* CCUG 32704^T with a bootstrap value of 99%. Strain CD276^T shared high 16S rRNA gene sequence similarities with *V. carniphilus* ATCC BAA-640^T (97.5%) and *V. fluvialis* CCUG 32704^T (97.3%) and lower sequence similarities were observed with the other type strains of the genus *Vagococcus* [*V. lutrae* CCUG 39187^T (96.2%), *V. elongatus* PPC9^T (95.6%), *V. salmoninarum* NCFB 2777^T (95.1%) and *V. fessus* M2661/98/1^T (94.2%)]. These results indicated that the group of five isolates was phylogenetically distinct from the six *Vagococcus* species already recognized.

Strain CD276^T was characterized phenotypically using conventional morphological and biochemical tests. All

phenotypic tests were performed at 30 °C except for growth tests at 10 and 45 °C. Cell morphology and motility were studied by phase-contrast microscopy. Motility was also examined in mannitol-nitrate mobility (MNM) medium (Bio-Rad), which was inoculated by stabbing the centre of the tube. Growth was studied in brain-heart infusion (BHI) broth with 0.1% glucose and bromocresol purple indicator at 10 and 45 °C or with 6.5% NaCl. Haemolysis was assessed on Columbia agar supplemented with 5% defibrinated horse blood (Bio-Rad) with 5–10% CO₂. Gas production from glucose was examined in MRS medium (Biokar Diagnostic) using Durham tubes sealed with melted petroleum jelly. Cells of strain CD276^T were Gram-stain-positive, catalase-negative, coccus-shaped bacteria (0.5–1 µm) that occurred singly, in pairs or in short chains, elongated in the direction of the chain, and were determined to be non-motile by microscopic and MNM medium observations. Colonies were small (0.5–1 mm), smooth and white on BHI agar at 30 °C. Strain CD276^T was facultatively anaerobic and produced lactic acid but not gas from glucose.

Sugar fermentation patterns and enzyme activities were determined for the five isolates with the API Rapid ID32S and API ZYM systems (bioMérieux), respectively, according to the manufacturer's instructions. All tests were carried out at least in duplicate. The detailed biochemical characteristics of strain CD276^T are presented in the species description and in Table 1. Some of the results for sugar fermentation and enzyme activities were variable between the isolates: CD279, CD285 and CD380 had identical

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence analysis, showing the relationships of strain CD276^T with the type strains of *Vagococcus* species and strains of selected *Enterococcus* species. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. Bar, 0.005 substitutions per nucleotide position.

profiles but differed from CD276^T in six reactions (α -cyclodextrin, α -chymotrypsin, α -glucosidase, arginine dihydrolase, glycyl-tryptophan arylamidase and leucine arylamidase) and from CD310 in eight reactions (raffinose, D-sorbitol, methyl β -D-glucopyranoside, α -cyclodextrin, β -glucosidase, β -mannosidase, arginine dihydrolase and glycyl-tryptophan arylamidase). Strain CD276^T was the second most reactive compared with the type strains of described *Vagococcus* species, having 15 positive reactions and one weakly positive reaction. *V. lutrae* CCUG 39187^T was the most reactive, having 18 positive reactions and five weakly positive reactions. Four reactions were positive for strain CD276^T exclusively and not for any other type strain: these were melezitose and raffinose acidification, arginine dihydrolase activity and acetoin production (Voges-Proskauer). Furthermore, strain CD276^T produced acid from several other carbohydrates, such as maltose, D-ribose, sucrose, trehalose and α -cyclodextrin, and displayed activity for acid phosphatase, glycyl-tryptophan arylamidase, naphthol-AS-BI-phosphohydrolase and pyroglutamic

acid arylamidase and weak activity for β -glucosidase, esterase (C4) and esterase lipase (C8).

An analysis of the peptidoglycan structure of strain CD276^T was carried out as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was applied instead of paper chromatography. Strain CD276^T possessed a cell-wall peptidoglycan of type A4 α L-Lys-D-Asp (type A11.31; http://www.dsmz.de/microorganisms/main.php?content_id=35).

For the quantitative analysis of cellular fatty acids, strain CD276^T was grown for 24 h on the medium described by Fischer & Arneith-Seifert (1998). Cells were harvested and cellular fatty acids were saponified, methylated and extracted as described by the instructions of the Sherlock Microbial Identification System (MIDI, 1999). Fatty acids were analysed by GC (model 6890; Hewlett Packard) and identified using the Microbial Identification software (Sasser, 1990). The predominant cellular fatty acids of strain CD276^T were *cis*-9 octadecenoic acid (18:1 ω 9;

Table 1. Phenotypic characteristics of isolates of *Vagococcus penaei* sp. nov. and the type strains of closely related *Vagococcus* species

Reference strains: 1, *V. fluvialis* CCUG 32704^T; 2, *V. salmoninarum* CCUG 33394^T; 3, *V. carniphilus* CCUG 46823^T; 4, *V. lutrae* CCUG 39187^T; 5, *V. fessus* CCUG 41755^T; 6, *V. elongatus* CCUG 51432^T. Data for reference strains were taken from the CCUG database (<http://www.ccug.se>). +, Positive; w, weakly positive; –, negative.

Characteristic	<i>Vagococcus penaei</i> sp. nov.					1	2	3	4	5	6
	CD276 ^T	CD279	CD285	CD380	CD310						
Acid from:											
Maltose	+	+	+	+	+	+	–	+	+	–	–
Melezitose	+	+	+	+	+	–	–	–	–	–	–
Raffinose	+	+	+	+	–	–	–	–	–	–	–
D-Ribose	+	+	+	+	+	+	–	+	+	–	–
D-Sorbitol	–	–	–	–	+	w	–	–	+	–	–
Sucrose	+	+	+	+	+	+	–	–	+	–	–
Trehalose	+	+	+	+	+	+	+	+	+	–	–
Methyl β -D-glucopyranoside	–	–	–	–	+	–	–	+	+	–	–
α -Cyclodextrin	+	–	–	–	+	+	–	+	+	–	–
Enzyme activities											
α -Chymotrypsin	–	+	+	+	+	w	+	–	+	+	–
α -Galactosidase	–	–	–	–	–	–	–	–	+	–	+
α -Glucosidase	–	+	+	+	+	w	–	–	+	–	–
β -Glucosidase	–	–	–	–	+	+	w	–	+	–	w
β -Mannosidase	–	–	–	–	+	w	–	–	+	–	–
Acid phosphatase	+	+	+	+	+	+	+	–	+	–	–
Alkaline phosphatase	–	–	–	–	–	–	w	w	w	–	w
Arginine dihydrolase	+	–	–	–	+	–	–	–	–	–	–
Esterase (C4)	+	+	+	+	+	w	w	–	w	w	w
Esterase lipase (C8)	+	+	+	+	+	+	w	–	+	w	w
Glycyl-tryptophan arylamidase	+	–	–	–	+	+	+	+	+	w	+
Leucine arylamidase	–	+	+	+	+	–	+	+	+	+	–
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	+	–	–	w	w	–
Pyroglutamic acid arylamidase	+	+	+	+	+	w	+	+	+	+	–
Acetoin production (Voges-Proskauer)	+	+	+	+	+	–	–	–	–	–	–

30.43 %) and hexadecanoic acid (16:0; 16.37 %). The complete fatty acid composition of strain CD276^T is shown in Table 2.

The analysis of chromosomal DNA restriction patterns for the five isolates was carried out by pulsed-field gel electrophoresis (PFGE) as described by Teixeira *et al.* (1997). Genomic DNA was digested with *Sma*I in agarose plugs and electrophoresed on 1.3 % agarose in TBE using the CHEF-DR III system (Bio-Rad). Band patterns were visualized and photographed under UV light. The PFGE profiles for the five isolates displayed two different banding patterns: one for isolates CD276^T and CD279 and another for isolates CD285, CD310 and CD380 (Fig. 2). The two patterns differed in eight bands. Thus, the two groups probably represent two distinct strains. The variation in PFGE profiles among the five isolates fulfils the recommendation of the International Committee for the Systematics of Prokaryotes for the basis of describing a species through the study of more than one strain (Stackebrandt *et al.*, 2002).

For the determination of G + C content, genomic DNA was prepared according to Gevers *et al.* (2001). The DNA G + C content was determined in triplicate by using HPLC (Mesbah *et al.*, 1989). The DNA G + C content of strain CD276^T was 35.4 mol%. This value is within the range (33.6–44.5 mol%) reported for the six recognized species of the genus *Vagococcus*.

DNA–DNA hybridization was carried out by the BCCM/ LMG Bacteria Collection (University of Gent, Belgium) with genomic DNA prepared according to a modification of the procedure described by Gevers *et al.* (2001). Hybridization was performed at 35 °C using the fluorometric method in duplicate, according to a modification of the method described by Ezaki *et al.* (1989). Table 3 shows

Table 2. Fatty acid content of strain CD276^T

Fatty acid	Systematic name	Proportion (%)
10:0	Decanoic acid	2.30
12:0	Dodecanoic acid	1.79
12:0 3-OH	3-Hydroxydodecanoic acid	0.64
14:0	Tetradecanoic acid	9.80
13:0 2-OH	2-Hydroxytridecanoic acid	0.51
15:1 F	13-Methyltetradecenoic acid isomer F	1.11
16:1 ω 9c	<i>cis</i> -9 Hexadecenoic acid	8.45
16:0	Hexadecanoic acid	16.37
iso-17:1 ω 9c	15-Methyl <i>cis</i> -9 hexadecenoic acid	1.06
iso-17:1 ω 5c	15-Methyl <i>cis</i> -5 hexadecenoic acid	1.27
18:1 ω 9c	<i>cis</i> -9 Octadecenoic acid	30.43
18:1 ω 7c	<i>cis</i> -7 Octadecenoic acid	4.20
18:0	Octadecanoic acid	6.29
19:1	Nonadecenoic acid	3.49
20:1 ω 9c	<i>cis</i> -9 Eicosenoic acid	5.88
20:1 ω 7c	<i>cis</i> -7 Eicosenoic acid	0.83

Fig. 2. Restriction patterns by PFGE of genomic DNA of isolates of *Vagococcus penaei* sp. nov. after *Sma*I digestion. M, Markers.

the mean DNA–DNA relatedness obtained between strain CD276^T and the type strains of the two phylogenetically most closely related species, *V. carniphilus* CIP 108561^T and *V. fluvialis* LMG 9464^T. The DNA–DNA hybridization experiments confirmed the phylogenetic grouping with the 16S rRNA gene sequence, with strain CD276^T having DNA–DNA relatedness of 14 and 18 % with *V. carniphilus* CIP 108561^T and *V. fluvialis* LMG 9464^T, respectively. These values were below the threshold of 70 % suggested for the delineation of species (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987) and indicated that strain CD276^T represents a novel genomic species.

Description of *Vagococcus penaei* sp. nov.

Vagococcus penaei [pe.na'e.i. N.L. gen. n. *penaei* of *Penaeus*, a zoological genus name, pertaining to the spoilage microbiota of cooked shrimp (*Penaeus vannamei*), from which the type strain was isolated].

Table 3. Mean DNA–DNA relatedness between strain CD276^T and its closest phylogenetic neighbours

Values are based on at least two hybridizations.

Strain	Hybridization (%) with DNA from:		
	1	2	3
1. <i>V. penaei</i> sp. nov. CD276 ^T	100	–	–
2. <i>V. carniphilus</i> CIP 108561 ^T	14 ± 2	100	–
3. <i>V. fluvialis</i> LMG 9464 ^T	18 ± 2	23 ± 0	100

Cells are Gram-stain-positive cocci, 0.5–1 µm in diameter, and occur singly, in pairs or in short chains, elongated in the direction of the chain. Cells are non-motile and non-spore-forming, facultatively anaerobic and catalase- and oxidase-negative. Colonies are small (up to 1 mm in diameter), smooth and white when grown on BHI agar at 30 °C. No haemolysis or pigment on Columbia agar supplemented with 5 % defibrinated horse blood. Lactic acid is produced from glucose fermentation, but gas is not produced. Grows at 10 °C but not at 45 °C. Does not grow with 6.5 % NaCl. With API Rapid ID32S, acid is produced from maltose, melezitose, raffinose, D-ribose, sucrose, trehalose and α -cyclodextrin, but not from L-arabinose, D-arabitol, lactose, D-mannitol, melibiose, D-sorbitol, D-tagatose, glycogen, methyl β -D-glucopyranoside or pullulan. Produces acid phosphatase, arginine dihydrolase, esterase (C4), esterase lipase (C8), glycyl-tryptophan arylamidase, naphthol-AS-BI-phosphohydrolase and pyroglutamic acid arylamidase, but not α -chymotrypsin, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -mannosidase, β -mannosidase, alkaline phosphatase, lipase (C14), alanyl-phenylalanyl-proline arylamidase, cystine arylamidase, leucine arylamidase, valine arylamidase, N-acetyl- β -glucosaminidase, trypsin or urease. Hippurate is not hydrolysed. Nitrate is not reduced. Acetoin is produced (Voges-Proskauer). The cell-wall peptidoglycan is type A4 α L-Lys-D-Asp and the predominant cellular fatty acids are *cis*-9 octadecenoic acid and hexadecanoic acid. The DNA G+C content of the type strain is 35.4 mol%.

The type strain is CD276^T (=LMG 24833^T =CIP 109914^T), isolated from the spoilage microbiota of cooked shrimp (*Penaeus vannamei*).

Acknowledgements

This study was supported by the Région Bretagne and the Région Pays de la Loire. We thank Florian Torselli for the molecular characterization. We are grateful to J. P. Euzéby for consultation regarding the species epithet. E. J. received a PhD grant awarded by Ifremer and ENITIAA.

References

- Al-Ahmad, A., Pelz, K., Schirrmeister, J. F., Hellwig, E. & Pukall, R. (2008). Characterization of the first oral *Vagococcus* isolate from a root-filled tooth with periradicular lesions. *Curr Microbiol* **57**, 235–238.
- Cole, J. R., Chai, B., Marsh, T. L., Farris, R. J., Wang, Q., Kulam, S. A., Chandra, S., McGarrell, D. M., Schmidt, T. M. & other authors (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- Collins, M. D., Ash, C., Farrow, J. A. E., Wallbanks, S. & Williams, A. M. (1989). 16S ribosomal ribonucleic acid sequence analyses of lactococci and related taxa. Description of *Vagococcus fluvialis* gen. nov., sp. nov. *J Appl Bacteriol* **67**, 453–460.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fischer, W. & Arneith-Seifert, D. (1998). D-Alanylcardiolipin, a major component of the unique lipid pattern of *Vagococcus fluvialis*. *J Bacteriol* **180**, 2950–2957.
- Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol Biol Evol* **14**, 685–695.
- Gevers, D., Huys, G. & Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol Lett* **205**, 31–36.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hashimoto, H., Noborisaka, R. & Yanagawa, R. (1974). Distribution of motile streptococci in man, animals and natural environment. *Nippon Saikingaku Zasshi* **29**, 387–393 (in Japanese).
- Hoyles, L., Lawson, P. A., Foster, G., Falsen, E., Ohlén, M., Grainger, J. M. & Collins, M. D. (2000). *Vagococcus fessui* sp. nov., isolated from a seal and a harbour porpoise. *Int J Syst Evol Microbiol* **50**, 1151–1154.
- Jaffrès, E., Sohier, D., Leroi, F., Pilet, M. F., Prévost, H., Joffraud, J. J. & Dousset, X. (2009). Study of the bacterial ecosystem in tropical cooked and peeled shrimps using a polyphasic approach. *Int J Food Microbiol* **131**, 20–29.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Lawson, P. A., Foster, G., Falsen, E., Ohlén, M. & Collins, M. D. (1999). *Vagococcus lutrae* sp. nov., isolated from the common otter (*Lutra lutra*). *Int J Syst Bacteriol* **49**, 1251–1254.
- Lawson, P. A., Falsen, E., Cotta, M. A. & Whitehead, T. R. (2007). *Vagococcus elongatus* sp. nov., isolated from a swine-manure storage pit. *Int J Syst Evol Microbiol* **57**, 751–754.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Michel, C., Pelletier, C., Boussaha, M., Douet, D. G., Lautraite, A. & Tailliez, P. (2007). Diversity of lactic acid bacteria associated with fish and the fish farm environment, established by amplified rRNA gene restriction analysis. *Appl Environ Microbiol* **73**, 2947–2955.
- Pot, B., Devriese, L. A., Hommez, J., Miry, C., Vandemeulebroecke, K., Kersters, K. & Haesebrouck, F. (1994). Characterization and identification of *Vagococcus fluvialis* strains isolated from domestic animals. *J Appl Bacteriol* **77**, 362–369.
- Ruiz-Zarzuela, I., de Bias, I., Girones, O., Ghittino, C. & Muazquiz, J. L. (2005). Isolation of *Vagococcus salmoninarum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum), broodstocks: characterization of the pathogen. *Vet Res Commun* **29**, 553–562.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schmidtke, L. M. & Carson, J. (1994). Characteristics of *Vagococcus salmoninarum* isolated from diseased salmonid fish. *J Appl Bacteriol* **77**, 229–236.
- Shewmaker, P. L., Steigerwalt, A. G., Morey, R. E., Carvalho, M. G. S., Elliott, J. A., Joyce, K., Barrett, T. J., Teixeira, L. M. & Facklam, R. R.

(2004). *Vagococcus carniphilus* sp. nov., isolated from ground beef. *Int J Syst Evol Microbiol* **54**, 1505–1510.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Teixeira, L. M., Carvalho, M. G. S., Merquior, V. L. C., Steigerwalt, A. G., Brenner, D. J. & Facklam, R. R. (1997). Phenotypic and genotypic

characterization of *Vagococcus fluvialis*, including strains isolated from human sources. *J Clin Microbiol* **35**, 2778–2781.

Valcheva, R., Ferchichi, M. F., Korakli, M., Ivanova, I., Gänzle, M. G., Vogel, R. F., Prévost, H., Onno, B. & Dousset, X. (2006). *Lactobacillus nantensis* sp. nov., isolated from French wheat sourdough. *Int J Syst Evol Microbiol* **56**, 587–591.

Wallbanks, S., Martinez-Murcia, A. J., Fryer, J. L., Phillips, B. A. & Collins, M. D. (1990). 16S rRNA sequence determination for members of the genus *Carnobacterium* and related lactic acid bacteria and description of *Vagococcus salmoninarum* sp. nov. *Int J Syst Bacteriol* **40**, 224–230.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.