Weissella ceti sp. nov., isolated from beaked whales (*Mesoplodon bidens*)

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During an investigation into the microbiota of beaked whales (*Mesoplodon bidens*), nine isolates were obtained from different organs of four animals. The isolates were Gram-positive-staining, catalase-negative, short rod-shaped or coccoid organisms. A phylogenetic analysis based on 16S rRNA gene sequences of these isolates allocated them to the genus *Weissella*, showing 96.3 % and 96.0 % 16S rRNA gene sequence similarity with *Weissella viridescens* NRIC 1536^{T}and *Weissella minor* NRIC 1625^T, respectively. On the basis of phenotypic, physiological and phylogenetic evidence, it is proposed that the new isolates from whales represent a novel species of the genus *Weissella*, *Weissella ceti* sp. nov. The type strain of *Weissella ceti* is 1119-1A-09^T (=CECT 7719^T=CCUG 59653^T).

Micro-organisms of the genus *Weissella* have been isolated from a wide variety of habitats such as soil, fresh vegetables and fermented foods or meat and meat products (Björkroth *et al.*, 2002; Magnusson *et al.*, 2002; De Bruyne *et al.*, 2010; Padonou *et al.*, 2010). In addition, some species have been isolated from human or animal sources. Thus, *Weissella cibaria* was isolated from human gall and faeces and *Weissella confusa* was isolated from faeces of children with bacteraemia (Green *et al.*, 1990), from liver transplants (Green *et al.*, 1991) and from the peritoneal fluid and abdominal wall of two patients (Riebel & Washington, 1990). In animals, *W. confusa* has been isolated from a primate (*Cercopithecus mona*) with a systemic infection (Vela *et al.*, 2003b), from an autopsied dog and from the ear of a dog with otitis (Björkroth *et al.*, 2002), and from intestines of farmed Asian seabass (*Lates calcarifer*) (Cai *et al.*, 1998; Rengpipat *et al.*, 2008). *W. cibaria* and *Weissella hellenica* have been isolated from the livers of canaries and from the ears of dogs with otitis and from the intestinal contents of flounder (*Paralichthys olivaceus*), respectively (Cai *et al.*, 1998; Björkroth *et al.*, 2002). Recently, members of the genus *Weissella* were isolated from diseased rainbow trout (*Oncorhynchus mykiss)* at a commercial fishery in China (Liu *et al.*, 2009), although they were not identified to species level. Other species of the genus *Weissella* isolated from animals have not been formally described. In this article, we report the phenotypic and phylogenetic characterization of an unusual *Weissella*-like organism isolated from stranded beaked whales (*Mesoplodon bidens*).

During an investigation into the microbiota of beaked whales, nine unidentified Gram-positive-staining, rod-shaped or coccoid organisms were recovered from muscle tissue (strains 1119-2B-09, 1121-2A-09 and 1122-2A-09), brain (1120-7A-09), kidney (1119-4A-09 and 1121-4A-09), lymph nodes (1121-8A-09), spleen (1119-1A-09^T and 1121-1A-09) of four different animals. None of these animals showed organic lesions associated with these unidentified bacteria after post-mortem studies (gross and histological examination). Strains were isolated on Columbia blood agar plates (bioMérieux) incubated for 24 h at 37 °C under both aerobic and anaerobic [with 4–10 % CO_2 using the GasPak Plus (BBL) system] conditions. On the basis of the phenotypic and phylogenetic results, a novel species of the genus *Weissella* is proposed.

The taxonomic position of the isolates from the stranded whales was investigated by 16S rRNA gene sequence analysis as described previously (Vela *et al.*, 2002). A large continuous fragment (approx. 1460 bp) of the 16S rRNA gene of the nine isolates was sequenced bidirectionally using universal primers pA (5′-AGAGTTTGATCCTGGCTCAG; positions 8–27, *Escherichia coli* numbering) and pH*

The GenBank/EMBL/DDBJ accession number 16S rRNA gene sequence of strain 1119-1A-09^T is FN813251.

(5′-AAGGAGGTGATCCAGCCGCA; positions 1541–1522, *E. coli* numbering). Comparative sequence analysis revealed that the isolates were phylogenetically identical (100 % sequence similarity). Sequence searches of GenBank using the FASTA program (Pearson, 1994) revealed that the unknown bacteria were members of the genus *Weissella*, being most closely related (99.2–99.5 % 16S rRNA gene sequence similarity) to isolates of the genus *Weissella* (GenBank accession nos. EU869289, EU869290, EU869291, EU869292, EU869293 and EU869294) recovered from diseased trout (Liu *et al.*, 2009). The high 16S rRNA gene similarity between the whale (as exemplified by strain 1119-1A-09^T) and trout isolates suggested that the latter could also represent isolates of the same novel species. Additional DNA–DNA hybridization experiments should be carried out to confirm this suggestion, which is beyond of the aim of this study focused on the formal description of a novel species of the genus *Weissella* from whales. For this reason, and because they have not been formally proposed as a novel species of the genus *Weissella* (Liu *et al.*, 2009), these six sequences were not included in the phylogenetic analysis.

The closest related species with validly published names were *Weissella viridescens* NRIC 1536^T and *Weissella minor* NRIC 1625^T with 96.3 % and 96.0 % 16S rRNA gene sequence similarity, respectively. Sequence similarity of strain 1119-1A-09^T with other recognized species of the genus *Weissella* was less than 95.7 %. These sequences and those of the type strains of all recognized species of the genus *Weissella* were retrieved from GenBank and aligned with the newly determined sequence using the SeqTools program (Rasmussen, 2002). Phylogenetic trees were constructed according to three different algorithms: neighbour-joining (Saitou & Nei, 1987) using the programs SeqTools and TreeView (Page, 1996; Rasmussen, 2002), maximum-parsimony using the software package MEGA (molecular evolutionary genetics analysis) version 4 (Kumar *et al.*, 2004) and maximum-likelihood using the PHYML software (Guindon & Gascuel, 2003). Genetic distances for the neighbour-joining algorithm were calculated by the Kimura two-parameter model (Kimura, 1980) and close-neighbour-interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis. The stability of the groupings was estimated by bootstrap analysis (1000 replications). The maximum-likelihood tree was calculated using the GTR model (Lanave *et al.*, 1984) based on the hill-climbing principle and estimated proportion of invariable sites, as well as the Gamma distribution parameter. The parameters in the PHYML program were as follows: input sequences were interleaved, with 500 non-parametric bootstrap analysis, GTR model of nucleotide substitution, four substitution rate categories and fixed Gamma distribution parameter (alpha =2.00). Phylogenetic trees obtained by using the neighbour-joining (Fig. 1) and other two methods (data not shown) revealed a clear affiliation of the unknown bacterium (as exemplified by strain 1119-1A-09^T) to the genus *Weissella*. It is evident from Fig. 1 that strain 1119-1A-09^T formed a distinct subline, clustering with a small subgroup of species embracing *W. minor*, *W. viridescens* and *Weissella halotolerans*. Bootstrap resampling revealed the affinity between the unknown bacterium isolated from the whales and the aforementioned species to be statistically significant (bootstrap value, 98 %). This, coupled with 16S rRNA gene sequence similarity values of <97 % between the isolates from the whales and the aforementioned species and all other recognized species of the genus, suggested that they represent a distinct species of the genus *Weissella* (Stackebrandt & Goebel, 1994).

The determination of the G+C content of the DNA for one representative isolate (strain 1119-1A-09^T) was performed at the DSMZ by using the HPLC method of Mesbah *et al.* (1989). The G+C content of the strain 1119-1A-09^T was 39.2 mol%.

The nine new isolates were Gram-stained and assessed for the presence of catalase. The haemolytic reaction was determined on Columbia agar containing 5 % defibrinated sheep blood (bioMérieux) incubated aerobically at 37 °C for 24 and 48 h (Facklam & Elliott, 1995). Determination of the growth in brain heart infusion broth (Difco) at pH 3.9 and at 15, 22, 30, 37 and 42 °C, with 3, 4.5 and 6.5 % (w/v) added NaCl in brain heart infusion broth (Difco) with the pH adjusted to 7.5 was performed as recommended by Facklam & Elliott (1995). The production of gas from glucose was assayed by growing the bacteria in MRS tubes containing Durham tubes. Dextran from sucrose was tested following the protocol of Schillinger & Lücke (1987). The isolates were biochemically characterized using the Rapid ID 32 Strep, API Coryne, API 50 CH and API ZYM systems (bioMérieux) according to the manufacturer's instructions. The API 50 CH strips, using the CHB suspension medium, were read after up to 7 days of incubation at 37 °C. The lactic isomer was determined enzymically using the DL-lactate test kit (Boehringer Mannheim) after growing the isolates for 96 h at 30 °C in MRS broth.

The isolates exhibited almost identical biochemical characteristics, except for the hydrolysis of arginine and production of alanine-phenylalanine-proline (isolates 1119-1A-09^T, 1119-2B-09, 1119-4A-09, 1120-7A-09 and 1122-2A-09 gave a positive reaction). The phenotypic characteristics that differentiate the new isolates from closely related recognized species are shown in Table 1.

The isolates from the stranded whales were characterized molecularly by pulsed-field gel electrophoresis according to the specifications of Vela *et al.* (2003a) using the restriction enzyme *Sma*I. The whale isolates displayed four different macrorestriction patterns (A–D; data not shown), indicating that they represented four separate strains. Macrorestriction patterns A and D were each isolated from one whale, while macrorestriction patterns B and C were both found in isolates taken from two different whales.

Overall, based on phenotypic and phylogenetic criteria, it is clear that the new catalase-negative bacterium represents a novel species of the genus *Weissella*, for which the name *Weissella ceti* sp. nov. is proposed.

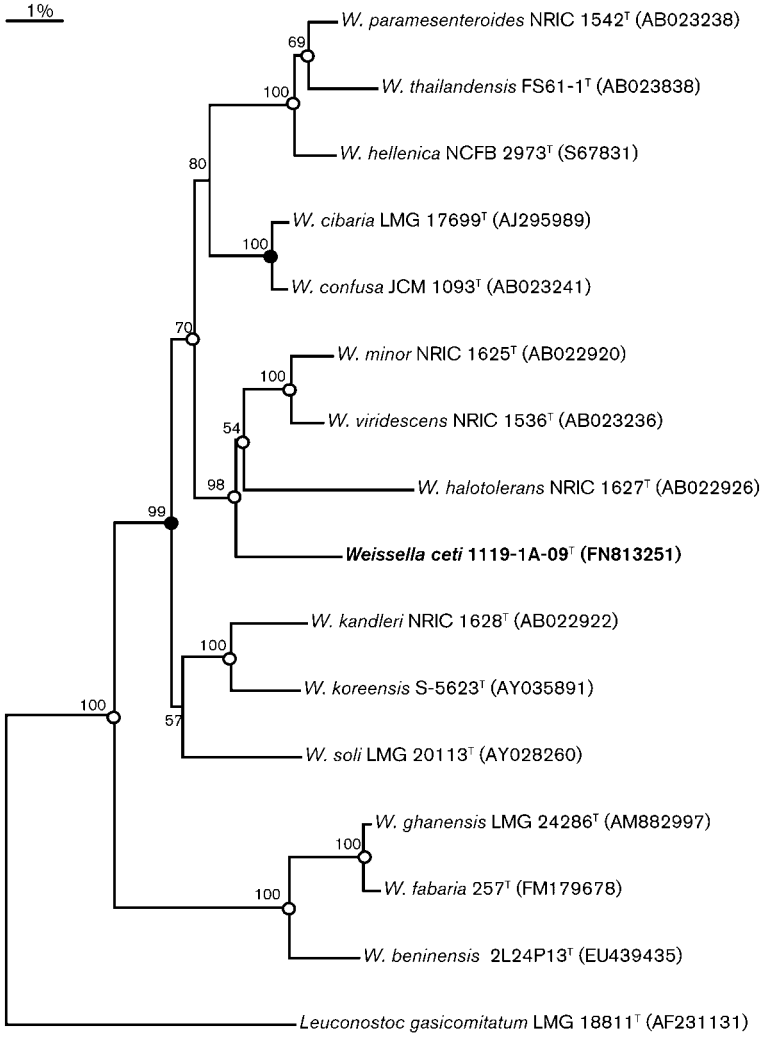

Fig. 1. Phylogenetic tree inferred from the 16S rRNA gene sequence comparison using the neighbour-joining method, showing the relationships of the new isolates with all recognized species of the genus *Weissella*. *Leuconostoc gasicomitatum* LMG 18811^T was used as an outgroup. Bootstrap values (expressed as a percentage of 1000 replications) >50 % are given at the branching points. Open circles indicate that the corresponding nodes (groupings) were also obtained in maximum-likelihood and maximum-parsimony trees. Filled circles indicate that the corresponding nodes (groupings) were also obtained on the parsimony tree. Bar, 1 % sequence divergence.

Description of *Weissella ceti* sp. nov.

Weissella ceti (ce.ti. L. gen. n. *ceti* of a whale).

Cells are Gram-positive-staining, non-spore-forming, short rod-shaped or coccoid (0.2 μm wide and 1.5 μm long) and occur singly or in pairs. Colonies on blood agar are small, circular and non-pigmented, 0.75–1.0 mm in diameter and are α-haemolytic at 37 °C. Cells are facultatively anaerobic, catalase-negative and non-motile. Cells are able to grow at 22, 30 and 37 °C, but do not grow at 15 or 42 °C. Growth occurs in broth containing 3.0–6.5 % NaCl, but not at pH 3.9. D and L lactic acid (D : L, 80 : 20) are produced as end products of glucose metabolism. Gas is not produced from glucose. Dextran is not formed from sucrose. Cells are able to produce acid from D-glucose, *N*-acetylglucosamine, D-ribose, trehalose and maltose, but not from D-xylose, D-galactose, D-fructose, D-mannose, L-rhamnose, amygdalin, arbutin, aesculin, salicin, cellobiose, lactose, sucrose, inulin, raffinose, starch, glycogen, pullulan, gentiobiose, methyl β-D-glucopyranoside, glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-arabitol, D-arabitol, D-mannitol, D-sorbitol, inositol, dulcitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, melezitose, turanose, D-lyxose, xylitol, D-fucose, L-fucose, 2-ketogluconate, 5-ketogluconate, cyclodextrin, L-arabinose, melibiose or tagatose. Alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and pyrazinamidase are detected. No activity is detected for β-glucuronidase, β-glucosidase, α-galactosidase, β-mannosidase, leucine arylamidase, β-galactosidase, α-mannosidase, α-fucosidase, esterase (C4), ester lipase (C8), lipase (C14), β-galactosidase, valine arylamidase, α-glucosidase, cystine arylamidase, trypsin, α-chymotrypsin, glycyl tryptophan arylamidase, *N*-acetyl-β-glucosaminidase or pyroglutamic acid arylamidase. Nitrate is not reduced. Acetoin is produced. Aesculin is hydrolysed, but not hippurate, gelatin or urea. Hydrolysis of arginine and production of alanine-phenylalanine-proline arylamidase are variable (the type strain, 1119-1A-09^T gives a positive result for both tests).

The type strain, 1119-1A-09^T (=CECT 7719^T=CCUG 59653^T), was isolated from the spleen of a beaked whale (*Mesoplodon bidens*). The full range of habitat is not

Table 1. Characteristics useful in differentiating *Weissella ceti* sp. nov. from closely related species of the genus *Weissella*

Strains: 1, *Weissella ceti* sp. nov. (nine strains); 2, *W. minor* CCUG 330668^T; 3, *W. viridescens* CCUG 30502^T; 4, *W. halotolerans* CCUG 33457^T. Phenotypic data for *W. ceti* and the other taxa were taken from this study and from Padonou *et al.* (2010). +, Positive; −, negative; NT, not tested; V, variable.

Characteristic	1	2	3	4
Hydrolysis of:				
Arginine	V*	−	−	+
Aesculin	+	+	−	−
Production of:				
Acetoin	+	+	+	−
Alanine-phenylalanine-proline arylamidase	V*	+	−	−
Alkaline phosphatase	+	−	−	−
α-Glucosidase	−	−	−	+
Esterase (C4)	−	−	−	+
Acid phosphatase	+	+	+	−
Naphthol-AS-BI-phosphohydrolase	+	−	+	−
Ester lipase (C8)	−	+	−	+
Pyroglutamic acid arylamidase	−	−	−	+
Production of acid from:				
D-Fructose	−	+	−	+
D-Mannose	−	+	−	+
D-Mannitol	−	+	−	+
Trehalose	+	+	−	−
D-Arabitol	−	+	−	−
Cellobiose	−	+	−	−
Sucrose	−	+	−	−
Methyl β-D-glucopyranoside	−	−	−	+
Melezitose	−	+	−	−
D-Ribose	+	+	−	+
D-Xylose	−	+	−	−
N-Acetylglucosamine	+	−	+	+
Dextran formation	−	−	NT	+

*Positive reaction for *W. ceti* 1119-1A-09^T.

known. The DNA G+C content of the type strain is 39.2 mol%.

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