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 1536T and Weissella minor NRIC 1625T, 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-09T (=CECT 7719T=CCUG 59653T).

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., 2002). Recently, members of the genus Weissella were isolated from diseased rainbow trout (Oncorhynchus mykiss) at a commercial fishery in China (Liu et al., 2011), 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-09T 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 % CO2 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’-AGAGTTTGATCTCGGCTCAG; positions 8–27, Escherichia coli numbering) and pH+.

The GenBank/EMBL/DDBJ accession number 16S rRNA gene sequence of strain 1119-1A-09T is FN813251.
1625T with 96.3 % and 96.0 % 16S rRNA gene sequence similarity using the software package MEGA (molecular evolution) (Saitou & Nei, 1987) using the programs SeqTools and (Rasmussen, 2002). Phylogenetic trees were constructed using the SeqTools program as the Gamma distribution parameter. The parameters in the principle and estimated proportion of invariable sites, as well as the 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-09T) to the genus Weissella. It is evident from Fig. 1 that strain 1119-1A-09T 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-09T) was performed at the DSMZ by using the HPLC method of Mesbah et al. (1989). The G + C content of the strain 1119-1A-09T 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-09T, 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 Smal. 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.
**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-xylene, D-galactose, D-fructose, D-mannose, L-rhamnose, amygdalin, arbutin, esculin, salicin, cellobiose, lactose, sucrose, inulin, raffinose, starch, glycogen, pullulan, gentiobiose, methyl β-D-glucopyranoside, glycerol, erythritol, D-arabinose, L-xylene, D-adenitol, methyl β-D-xylpyranoside, L-sorbose, L-arabitol, D-arabitol, D-mannitol, D-sorbitol, inositol, dulcitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, melezitose, turanose, D-lyxose, xyitol, L-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, β-mannosidase, α-fucosidase, esterase (C4), ester lipase (C8), lipase (C14), β-galactosidase, valine arylamidase, x-galactosidase, cystine arylamidase, trypsin, x-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 alane-phenylalanine-proline arylamidase are variable (the type strain, 1119-1A-09T gives a positive result for both tests).

The type strain, 1119-1A-09T (=CETC 7719T=CCUG 59653T), 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*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>Hydrolysis of:</td>
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<tr>
<td>Arginine</td>
<td>v*</td>
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<tr>
<td>Aesculin</td>
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<tr>
<td>Production of:</td>
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<tr>
<td>Acetoin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Alanine-phenylalanine-proline arylamidase</td>
<td>v*</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
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<td>α-Glucosidase</td>
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<td>Esterase (C4)</td>
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<td>+</td>
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<tr>
<td>Acid phosphatase</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
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<td>+</td>
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<tr>
<td>Ester lipase (C8)</td>
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<td>Pyroglycamic acid arylamidase</td>
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</table>

Production of acid from:

- d-Fructose: + + + -
- d-Mannose: - - - +
- d-Mannitol: + + - +
- Trehalose: + + - -
- d-Arabinol: - - + -
- Cellobiose: - + - -
- Sucrose: - - - -
- Methyl β-d-glucopyranoside: - - - -
- Melezitose: - - - -
- d-Ribose: + + - -
- d-Xylose: - - - -
- N-Acetylg glucosamine: + - + +
- Dextran formation: - NT +

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

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

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**References**


