Isobaculum melis gen. nov., sp. nov., a Carnobacterium-like organism isolated from the intestine of a badger

Matthew D. Collins,1 Roger A. Hutson,1 Geoffrey Foster,2 Enevold Falsen3 and Norbert Weiss4

Author for correspondence: Matthew D. Collins. Tel: +44 118 935 7000. Fax: +44 118 926 7917. e-mail: m.d.collins@reading.ac.uk

Phenotypic and phylogenetic studies were performed on a hitherto undescribed facultatively anaerobic, catalase-negative, Gram-positive rod-shaped organism, strain M577-94T, isolated from the small intestine of a dead badger. It resembled carnobacteria in terms of its long-chain cellular fatty acid composition, but differed markedly from the latter in possessing a cell-wall murein based on L-lysine (type L-Lys–L-Thr–Gly). Comparative 16S rRNA gene sequencing showed that the unknown bacterium represents a new line closely related to, albeit distinct from, the genera Carnobacterium and Desemzia. On the basis of phylogenetic and phenotypic evidence, it is proposed that strain M577-94T be classified as Isobaculum melis gen. nov., sp. nov. The type strain of Isobaculum melis is CCUG 37660T (= DSM 13760T).

Keywords: Isobaculum melis, 16S rRNA, taxonomy, phylogeny

The genus Carnobacterium was proposed as the genus accommodating the species Lactobacillus divergens and Lactobacillus piscicola and a number of atypical lactobacilli which had been isolated from refrigerated meats (Collins et al., 1987). The genus originally contained four species (Carnobacterium divergens, Carnobacterium piscicola, Carnobacterium gallinarum and Carnobacterium mobile). Three further species, Carnobacterium funditum and Carnobacterium alterfunditum, originating from Antarctic lakes (Franzmann et al., 1993), and Carnobacterium inhibens, isolated from Atlantic salmon (Jöborn et al., 1999), have subsequently been assigned to the genus. Carnobacteria resemble lactobacilli in consisting of Gram-positive, catalase-negative, fermentative, asporogenous rod-shaped organisms. Molecular genetic studies, however, have revealed that carnobacteria are only remotely related to lactobacilli and in fact show a closer phylogenetic affinity with several catalase-negative coccos-shaped organisms such as enterococci, vagococci and Lactosphaera pasteurii (e.g. Collins et al., 1991, 1997, 1998; Stackebrandt et al., 1999). During the course of an investigation of unusual Gram-positive catalase-negative organisms associated with animals, we have characterized a hitherto unknown non-spore-forming rod-shaped bacterium, isolated from a badger, which somewhat resembles carnobacteria. Here we report the results of a polyphasic taxonomic study on the unknown rod, and propose that it be classified as a new genus and species, namely Isobaculum melis.

Strain M577-94T (= CCUG 37660T = DSM 13760T) was isolated from the small intestine of a dead badger and was cultured on Columbia agar supplemented with 5% defibrinated horse blood in air at 37 °C. The strain was characterized by using conventional physiological tests (Facklam & Elliot, 1995) and also by using the API rapid ID32Strep, API CORYNE and API ZYM systems according to the manufacturer’s instructions (API bioMérieux). The cell-wall murein structure of strain CCUG 37660T was determined by the methods of Schleifer & Kandler (1972) except that ascending TLC on cellulose sheets was used. Long-chain cellular fatty acids were examined using the MIDI (MIDI) system. The 16S rRNA gene of the isolate was amplified by a PCR and directly sequenced using a Taq dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolate were determined by performing database searches. A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR.
produced acid from conventional methods (Facklam & Elliot, 1995). It and did not hydrolyse hippurate, starch or urea using midase-positive and leucine aminopeptidase-negative, positive bile aesculin reaction, was pyrrolydonyl arylamidase, in deMan, Rogosa and Sharpe broth, gave a

The unknown strain CCUG 37660 (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 strain CCUG 37660 originating from the small intestine of a dead badger consisted of Gram-positive asporogenous rods which were non-motile. The organism was facultatively anaerobic and catalase- and oxidase-negative. It produced growth at 10 °C but not 45 °C and did not grow in broth containing 6-5% NaCl. The isolate failed to produce gas in deMan, Rogosa and Sharpe broth, gave a positive bile aesculin reaction, was pyrrolydonyl arylamidase-positive and leucine aminopeptidase-negative, and did not hydrolyse hippurate, starch or urea using conventional methods (Facklam & Elliot, 1995). It produced acid from α-glucose, glycerol, d-ribose and trehalose but not from L-arabinose, inulin, lactose, maltose, melezitose, melibiose, d-raffinose, sorbitol, sorbose or sucrose in conventional heart infusion base medium (Facklam & Elliot, 1995). The organism did not utilize pyruvate and was Voges–Proskauer-negative. When API kits were used, the isolate produced acid from α-glucose, d-arabinose, d-ribose and trehalose but not from L-arabinose, inulin, lactose, maltose, melezitose, melibiose, d-riaffinose, sorbitol, sorbose or sucrose in conventional heart infusion base medium (Facklam & Elliot, 1995). The organism did not utilize pyruvate and was Voges–Proskauer-negative. When API kits were used, the isolate produced acid from α-glucose, d-arabinose, d-ribose and trehalose but not from L-arabinose, inulin, lactose, maltose, melezitose, melibiose, d-riaffinose, sorbitol, sorbose or sucrose in conventional heart infusion base medium (Facklam & Elliot, 1995). The organism did not utilize pyruvate and was Voges–Proskauer-negative.

As observed for acid phosphatase (weak reaction), arginine dihydrolase, esterase C-4 (weak reaction), ester lipase C-8 (weak reaction), β-glucosidase, pyrogulat-}

unsaturated types with C14:0 (11%), C16:0 (32%), C18:0 (15%) and C18:1ω9c (30%) predominating. The DNA base composition of the organism was 39 mol% G+C, and cell wall analysis revealed the presence of an A3z murein (type L-Lys–L-Thr–Gly). To ascertain the phylogenetic relationships of the unidentified rod, its 16S rRNA gene sequence was determined and subjected to a comparative analysis. Sequence searches showed that the rod-shaped organism was a member of the Clostridium subphylum of the Gram-positive bacteria and displayed highest sequence relatedness to the genus Carnobacterium (94-95% sequence similarity) and Desemzia incerta (94.5%). A tree showing the position of the rod-shaped bacterium in relation to its nearest phylogenetic relatives is shown in Fig. 1. The unknown rod formed a distinct line branching near to the base of the genera Carnobacterium and Desemzia. It is evident from the results of the polyphasic taxonomic study that the unidentified asporogenous rod-shaped organism isolated from a badger represents a hitherto unknown taxon. Phylogenetically, the bacterium forms a distinct subline close to the genera Carnobacterium and Desemzia. Bootstrap resampling showed that the unknown rod did not possess a statistically significant association with either of these taxa. The badger bacterium was also found to be phenotypically very distinct. For example, the presence of large amounts of oleic acid meant that the unknown organism resembled the carnobacteria, whereas many other catalase-negative, asporogenous rod-shaped taxa such as lactobacilli and D. incerta contain cis-vaccenic acid (Collins et al., 1987). However, the presence of an A3z murein based on L-lysine (type L-Lys–L-Thr–Gly) in the badger bacterium represents a marked difference with respect to the wall mureins of members of the genus Carnobacterium, which are invariably based on meso-diaminopimelic acid. Similarly, the cell wall composition reinforces the difference between the badger bacterium and D. incerta, as the latter contains the L-lysine–D-glutamic acid murein type (Stackebrandt et al., 1999). The 16S rRNA tree branching pattern together with these marked chemotaxonomic differences strongly support the assignment

**Fig. 1.** Unrooted tree showing the phylogenetic relationships of *Isobaculum meles* sp. nov. and some other low-G+C Gram-positive bacteria. The tree constructed using the neighbour-joining method was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at branching points.
of the unknown rod to a separate genus. In addition to the aforementioned chemical differences, the biochemical reactions (API rapid ID32Strep profile 30120310040 and API CORYNE profile 4140300) of the badger bacterium readily serve to distinguish it from all described *Carnobacterium* species, *D. incerta* and other asporogenous rod-shaped taxa. Therefore, on the basis of the phenotypic and phylogenetic evidence presented we propose that the unidentified rod-shaped organism be classified as a new genus and species, namely *Isobaculum melis*. Characteristics that are useful in distinguishing *I. melis* from *D. incerta* and *Carnobacterium* species are shown in Table 1.

### Table 1. Characteristics that differentiate *Isobaculum melis* from its nearest phylogenetic relatives

<table>
<thead>
<tr>
<th>Strains</th>
<th>Characteristics</th>
<th>DNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, <em>Isobaculum melis</em> CCUG 37660&lt;sup&gt;9&lt;/sup&gt; (&lt;em&gt;DSM 13760&lt;/em&gt;)&lt;sup&gt;9&lt;/sup&gt;</td>
<td>44 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>2, <em>Desemzia incerta</em> CCUG 38799&lt;sup&gt;5&lt;/sup&gt;</td>
<td>44 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>3, <em>Carnobacterium alterfundium</em> CCUG 34643&lt;sup&gt;3&lt;/sup&gt;</td>
<td>39 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>4, *Carnobacterium diversog NCDO 2763&lt;sup&gt;3&lt;/sup&gt;</td>
<td>39 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>5, <em>Carnobacterium funditum</em> CCUG 34644&lt;sup&gt;5&lt;/sup&gt;</td>
<td>39 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>6, <em>Carnobacterium gallinarum</em> CCUG 30095&lt;sup&gt;7&lt;/sup&gt;</td>
<td>39 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>7, <em>Carnobacterium mobile</em> CCUG 30096&lt;sup&gt;7&lt;/sup&gt;</td>
<td>39 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>8, <em>Carnobacterium piscicola</em> CCUG 34645&lt;sup&gt;9&lt;/sup&gt;</td>
<td>39 mol%</td>
<td>39 mol%</td>
</tr>
<tr>
<td>9, <em>Carnobacterium inhibens</em> CCUG 31728&lt;sup&gt;7&lt;/sup&gt;</td>
<td>39 mol%</td>
<td>39 mol%</td>
</tr>
</tbody>
</table>

Biochemical tests were done with the API rapid ID32S system. –, Negative test result; +, positive test result. MDpm, meso-Diaminopimelic acid.

**Description of Isobaculum melis sp. nov.**


Cells consist of non-spor-forming, non-motile rods. Cells mainly stain Gram-positive although some cells have a tendency to decolorize easily and appear Gram-negative. Non-pigmented and non-haemolytic. Facultatively anaerobic, oxidase- and catalase-negative. Grows at 10 °C but not at 45 °C or in 6.5% NaCl. Gas is not produced in deMan, Rogosa and Sharpe broth. (l(+))Lactic acid and acetic acid are the major end products of glucose metabolism. In conventional heart infusion base medium, acid is produced from D-glucose, glycerol, δ-ribose and trehalose. Acid is not produced from arabinose, inulin, lactose, maltose, melezitose, melibiose, D-raffinose, sorbitol, sorbose or sucrose. When API systems are used, acid is produced from δ-glucose, trehalose and ribose but not from L-arabinose, d-arabitol, cyclodextrin, glyoxin, lactose, melibiose, melezitose, maltose, mannitol, pullulan, D-raffinose, sorbitol, sucrose, δ-tagatose, D-xylose or methyl β-d-glucopyranoside. Acid phosphatase (weak reaction), arginine dihydrolase, ester lipase C-8 (weak reaction), esterase C-4 (weak reaction), β-glucosidase, pyruvate- and phosphoaminidase and β-mannosidase are produced. Alanine phenylalanine proline arylamidase, chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, glycine tryptophan arylamidase, z-mannosidase, leucine arylamidase, lipase C-14, pyrazinamidase, tlypsin, urease and valine arylamidase are not produced. N-Acetyl-β-
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glucosaminidase and alkaline phosphatase activities may or may not be present. Aesculin is hydolysed but hippurate, starch and gelatin are not hydrolysed. Nitrate is not reduced to nitrite. Voges–Proskauer-negative. Pyruvate is not utilized. Acid is produced in litmus milk, but clotting is not observed. Sensitive to vancomycin (30 µg disc). The major long-chain fatty acids are C16:0, C18:0 and C18:1ω9c. Menaquinones are absent. The cell wall contains L-lysine of the L-Lys–L-Thr–Gly type. The G+C content of the DNA is 39 mol%. Isolated from the small intestine of a badger killed in a road accident. Habitat unknown. The type strain is CCUG 37660T (= DSM 13760T).

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References


