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***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 Weiss⁴¹ School of Food Biosciences, Whiteknights, University of Reading, Reading RG6 6AP, UK² SAC Veterinary Science Division, Drummondhill, Inverness, UK³ Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Göteborg Sweden⁴ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

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Phenotypic and phylogenetic studies were performed on a hitherto undescribed facultatively anaerobic, catalase-negative, Gram-positive rod-shaped organism, strain M577-94^T, 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 M577-94^T be classified as *Isobaculum melis* gen. nov., sp. nov. The type strain of *Isobaculum melis* is CCUG 37660^T (= DSM 13760^T).

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 inhi-bens*, 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 coccus-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 un-

known non-spore-forming rod-shaped bacterium, isolated from a badger, which somewhat resembles carnobacteria. Here we report the results of a poly-phasic taxonomic study on the unknown rod, and propose that it be classified as a new genus and species, namely *Isobaculum melis*.

Strain M577-94^T (= CCUG 37660^T = DSM 13760^T) 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 37660^T 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

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 37660^T (= DSM 13760^T) is AJ302648.

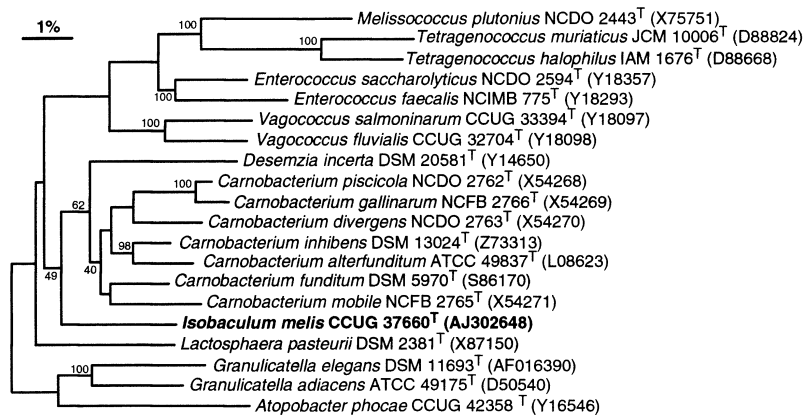


Fig. 1. Unrooted tree showing the phylogenetic relationships of *Isobaculum melis* sp. nov. and some other low-G+C Gram-positive bacteria. The tree constructed using the neighbour-joining method was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at branching points.

(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^T 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 D-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 D-glucose, trehalose and D-ribose but not from L-arabinose, D-arabitol, cyclodextrin, glycogen, lactose, melibiose, melezitose, maltose, mannitol, pululan, D-raffinose, sorbitol, sucrose, D-tagatose, D-xylose or methyl β -D-glucopyranoside. Activity was observed for acid phosphatase (weak reaction), arginine dihydrolase, esterase C-4 (weak reaction), ester lipase C-8 (weak reaction), β -glucosidase, pyroglutamic acid arylamidase, pyrrolydonyl arylamidase, phosphoaminidase and β -mannosidase but not for alanine phenylalanine proline arylamidase, chymotrypsin, cystine arylamidase, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucuronidase, glycine tryptophan arylamidase, α -mannosidase, leucine arylamidase, lipase C-14, pyrazinamidase, trypsin, urease or valine arylamidase. Different results were obtained for N-acetyl- β -glucosaminidase: activity was detected with the API rapid ID32Strep system but not with the API ZYM or API CORYNE systems. The long-chain cellular fatty acids of the unidentified isolate consisted of straight-chain saturated and mono-

unsaturated types with C14:0 (11%), C16:0 (32%), C18:0 (15%) and C18:1 ω 9c (30%) predominating. The DNA base composition of the organism was 39 mol% G+C, and cell wall analysis revealed the presence of an A3 α 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 A3 α 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

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

Strains: 1, *Isobaculum melis* CCUG 37660^T (= DSM 13760^T); 2, *Desemzia incerta* CCUG 38799^T; 3, *Carnobacterium alterfundium* CCUG 34643^T; 4, *Carnobacterium divergens* NCDO 2763^T; 5, *Carnobacterium funditum* CCUG 34644^T; 6, *Carnobacterium gallinarum* CCUG 30095^T; 7, *Carnobacterium mobile* CCUG 30096^T; 8, *Carnobacterium piscicola* CCUG 34645^T; 9, *Carnobacterium inhibens* CCUG 31728^T. Biochemical tests were done with the API rapid ID32S system. –, Negative test result; +, positive test result. mDpm, meso-Diaminopimelic acid.

Test	1	2	3	4	5	6	7	8	9
Acid from:									
Maltose	–	+	+	+	+	+	–	+	+
Mannitol	–	–	–	–	–	–	–	–	+
Ribose	+	+	–	+	–	+	–	+	+
Sucrose	–	+	+	+	+	+	+	+	+
Production of:									
Arginine dihydrolase	+	–	–	+	–	+	+	+	–
Glycine tryptophan arylamidase	–	+	–	+	+	+	–	+	–
Pyroglutamic acid arylamidase	+	+	–	+	–	+	+	+	–
<i>N</i> -Acetyl- β -glucosaminidase	+	+	–	+	–	+	–	+	–
Voges-Proskauer	–	–	–	+	–	+	–	+	–
Murein type	Lys-L-Thr-Gly Lys-D-Glu m-Dpm m-Dpm m-Dpm m-Dpm m-Dpm m-Dpm m-Dpm								

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.

Description of *Isobaculum* gen. nov.

Isobaculum (iso.bac'u.lum. Gr. adj. *isos* alike, similar; L. neut. n. *baculum* small rod; N.L. neut. n. *Isobaculum* the one like a stick or rod).

Cells consist of non-spore-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 and oxidase- and catalase-negative. Grows at 10 °C but not at 45 °C or in broth containing 6.5% NaCl. Gas is not produced in deMan, Rogosa and Sharpe broth. Acetate and lactate are the end products of glucose metabolism. Aesculin is hydrolysed but starch, gelatin and urea are not hydrolysed. Arginine dihydrolase, pyrrolydonyl arylamidase and β -glucosidase are produced. Pyruvate is not utilized. Voges-Proskauer-negative. Sensitive to vancomycin. Contains an A3 α murein based on L-lysine. Menaquinones are absent. The long-chain cellular fatty acids are predominantly of the straight-chain saturated and monounsaturated types. The G + C content of the

DNA is 39 mol%. The type species of the genus is *Isobaculum melis*.

Description of *Isobaculum melis* sp. nov.

Isobaculum melis (me'lis. L. fem. n. *meles* badger, L. gen. fem. n. *melis* of the badger).

Cells consist of non-spore-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, D-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 D-glucose, trehalose and ribose but not from L-arabinose, D-arabitol, cyclodextrin, glycogen, lactose, melibiose, melezitose, maltose, mannitol, pullulan, D-raffinose, sorbitol, sucrose, D-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, pyroglutamic acid arylamidase, 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, α -mannosidase, leucine arylamidase, lipase C-14, pyrazinamidase, trypsin, urease and valine arylamidase are not produced. *N*-Acetyl- β -

glucosaminidase and alkaline phosphatase activities may or may not be present. Aesculin is hydrolysed 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 C18:1 ω 9c. 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 37660^T (= DSM 13760^T).

Acknowledgements

We are grateful to Richard R. Facklam for performing some conventional biochemical tests, and to Hans Trüper for help with the derivation of the genus and species names. The SAC Veterinary Science Division receives funding from the Scottish Executive Rural Affairs Department.

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