Mycetocola reblochoni sp. nov., isolated from the surface microbial flora of Reblochon cheese

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Four Gram-positive, aerobic, non-sporulating, rod-shaped bacteria isolated from the surface microflora of Reblochon cheese at the late stage of ripening had chemotaxonomic properties characteristic of members of the family *Microbacteriaceae*. The isolates had virtually identical SDS-PAGE whole-organism protein patterns, shared many chemical and phenotypic characteristics and formed an independent branch in the *Microbacteriaceae* 16S rRNA gene tree that was most closely related to the type strains of *Mycetocola* species. The new isolates had chemotaxonomic properties consistent with their classification in the genus *Mycetocola* but were readily distinguished from recognized members of this taxon based on DNA–DNA relatedness, whole-organism protein and phenotypic data. The combined genotypic and phenotypic data indicate that the isolates should be classified in the genus *Mycetocola* as members of a novel species, for which the name *Mycetocola reblochoni* sp. nov. is proposed. The type strain is LMG 22367^T (=R-20377^T =BRB-1L41^T =DSM 18580^T).

The largely undefined microbial communities present on the surfaces of smear-ripened cheeses (Eliskases-Lechner & Ginzinger, 1995; Valdes-Stauber *et al.*, 1997) include members of novel bacterial taxa. *Corynebacterium casei* and *Microbacterium gubbeenense*, for instance, were isolated from the surface of the Irish farmhouse surface-ripened cheese Gubbeen (Brennan *et al.*, 2001a, b, 2002), and *Arthrobacter bergerei* and *Arthrobacter arilaitensis* were isolated from different smear-ripened cheeses (Irlinger *et al.*, 2005). During the course of a study into the biodiversity of the surface flora of Reblochon cheese, a traditional smear-ripened cheese manufactured from cow's milk in a restricted area of the northern French Alps, a homogeneous group of coryneform strains were isolated and provisionally assigned to the family *Microbacteriaceae* Park *et al.* 1995 emend. Rainey *et al.* 1997 (Park *et al.*, 1993; Stackebrandt *et al.*, 1997). Representative members of this group plus the type strains of recognized *Mycetocola* species were the subject of a polyphasic taxonomic study, which showed that the Reblochon strains represent a novel species of the genus *Mycetocola*.

At the time of writing, the family *Microbacteriaceae* comprises 21 genera with validly published names, including the recently described genera *Frondicola* and *Labedella* (Lee, 2007). The family includes coccoid, rod-shaped and mycelium-forming strains that have a β-type cell-wall peptidoglycan and unsaturated menaquinones. The constituent genera can be distinguished readily based on chemotaxonomic characteristics, such as the diamino acid of the cell-wall peptidoglycan (γ-aminobutyric acid, 2,4-diaminobutyric acid, lysine and ornithine) and fatty acid, menaquinone and polar lipid components (Evtushenko & Takeuchi, 2006; Sheridan *et al.*, 2003; Manaia *et al.*, 2004; Lee, 2007).

Bacteria were isolated from Reblochon cheese following suspension and homogenization (stomacher; Interscience) of samples of the red smear surface in sodium citrate (2 %,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 22367^T, LMG 23021, LMG 23022 and LMG 23020 are DQ062097–DQ062100, respectively.

w/v). After tenfold dilutions and plating onto plate count agar (PCA; Biokar Diagnostic) supplemented with 3 % (w/v) sodium chloride, cultures were isolated, purified and screened by using rep-PCR. They were then grown for 1–2 days at 30 °C on trypticase soy broth (TSB; Difco) containing 1.5 % agar (Oxoid). DNA isolation and electrophoresis were performed according to Gevers *et al.* (2001) and PCR was performed with the primer BOXA1R following Versalovic *et al.* (1994). Band patterns were analysed by using Pearson's product–moment correlation coefficient and UPGMA cluster analysis with Bionumerics software (Applied Maths), and were then compared with a database consisting of type and representative strains of the most common species present on red smear cheeses (data not shown). A homogeneous cluster consisting of eight isolates (LMG 22367^T, LMG 23020, LMG 23021, LMG 23022, R-20391, R-20398, R-20413 and R-20423) remained unidentified; the taxonomic position of these isolates is the subject of the present study.

The eight strains were isolated from Reblochon cheeses manufactured in September 2002 in three different farmhouses. The cheeses were made from raw milk without the addition of starter surface microflora and were ripened on wooden shelves. All of the isolates were recovered at the late stage of ripening, i.e. after about 15 days. Four representative strains, LMG 22367^T, LMG 23020, LMG 23021 and LMG 23022, were selected for further taxonomic study. The reference type strains used in the present study, *Mycetocola lacteus* LMG 22187^T, *Mycetocola saprophilus* LMG 22188^T and *Mycetocola tolaasinivorans* LMG 22189^T, were obtained from the BCCM/LMG Bacteria Collection (http://www.belspo.be/bccm/lmg.htm).

The phylogenetic position of the representative isolates was determined by 16S rRNA gene sequence analysis. Cells of the organisms were grown in brain heart infusion broth (Difco) for 5 days at 30 °C, and the biomass was checked for purity, harvested by centrifugation, washed in NaCl/EDTA buffer (0.1 M EDTA, 0.1 M NaCl, pH 8.0) and stored at −20 °C until required. Genomic DNA, extracted as described by Sambrook & Russell (2001), was used as template for PCR amplification and sequencing following the procedure of Kim *et al.* (1998). The resultant almost-complete 16S rRNA gene sequences (1541 nt) were aligned manually with corresponding sequences of representatives of the genera classified in the family *Microbacteriaceae*, retrieved from the RDP and GenBank databases, by using the pairwise alignment option and 16S rRNA secondary structure information held in the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/).

Phylogenetic trees were inferred by using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYDIT program. Evolutionary distance matrices for the least-squares and neighbour-joining algorithms were generated by using the distance model of Jukes & Cantor (1969). The resultant unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset by using the SEQBOOT and CONSENSE options from the PHYLIP package (Felsenstein, 1993). The resultant phylogenetic analyses showed that the new isolates formed an independent phyletic line among the actinomycete genera classified in the family *Microbacteriaceae* (Stackebrandt *et al.*, 1997; Lee, 2007) (Fig. 1). The new isolates shared 16S rRNA gene sequence similarities of 99.9–100 % and were most closely related to the type strains of *Mycetocola* species, namely *Mycetocola lacteus* LMG 22187^T, *Mycetocola saprophilus* LMG 22188^T and *Mycetocola tolaasinivorans* LMG 22189^T. The relationship between the new isolates and the *Mycetocola* strains was supported by all of the tree-making algorithms and by a high level (80 %) of bootstrap support in the neighbour-joining analysis. *Mycetocola* strains were not included in the initial rep-PCR screening as members of this taxon have not been found previously amongst the surface flora of red smear cheeses.

SDS-PAGE of whole-organism proteins, a method routinely used for species delineation, was carried out as a screening method to reveal relationships between the representative isolates and the *Mycetocola* reference strains. *Mycetocola lacteus* LMG 22187^T, *Mycetocola saprophilus* LMG 22188^T and *Mycetocola tolaasinivorans* LMG 22189^T and the Reblochon isolates were cultivated as indicated for the rep-PCR analyses. Whole-organism protein extracts were prepared and SDS-PAGE was performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of protein profiles, and numerical analyses were carried out by using Gelcompar software package, versions 3.1 and 4.0 (Applied Maths). The Reblochon isolates formed a homogeneous cluster that was sharply separated from the *Mycetocola* reference strains (Fig. 2).

The DNA G+C content of strain LMG 22367^T was determined. Cells were cultivated in TSB for 24 h at 28 °C. DNA was extracted from 0.75–1.25 g wet weight of cells by using the protocol described by Pitcher *et al.* (1989) with the following modifications: the washed cell pellet was resuspended and lysed in a buffer (10 mM Tris/HCl, 100 mM EDTA, pH 8.0) containing RNase (200 μg ml^{-1}; Sigma), mutanolysin (100 U ml^{-1}; Sigma) and lysozyme (25 mg ml^{-1}; SERVA) for 1 h at 37 °C. Before the addition of GES reagent, proteinase K (200 μg ml^{-1}; Merck) was added to the mixture for 15 min and the DNA was degraded enzymically into nucleosides, as described by Mesbah *et al.* (1989). The nucleoside mixture was separated by HPLC by using a Waters SymmetryShield C8 column maintained at 37 °C with 0.02 M $NH_4H_2PO_4$ (pH 4.0) and 1.5 % acetonitrile as the solvent. Non-methylated λ-phage DNA (Sigma) was used as the calibration reference. The DNA G+C content of strain LMG 22367^T was 70 mol%, a value higher than the 64–65 mol% reported for recognized members of the genus *Mycetocola* (Tsukamoto *et al.*, 2001).

DNA–DNA hybridizations were performed between strain LMG 22367^T and *Mycetocola lacteus* LMG 22187^T,

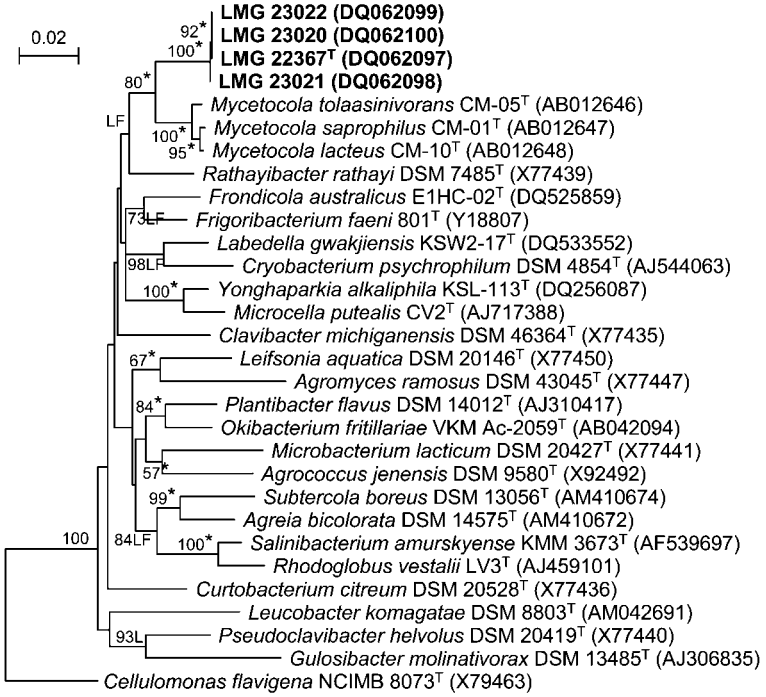

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing the position of isolates LMG 22367^T, LMG 23020, LMG 23021 and LMG 23022 in the *Microbacteriaceae* 16S rRNA gene tree. Asterisks indicate branches of the tree that were also found by using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. L and F respectively indicate branches that were recovered by using the maximum-likelihood and least-squares methods. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0.02 substitutions per nucleotide position.

Mycetocola saprophilus LMG 22188^T and *Mycetocola tolaasinivorans* LMG 22189^T by using genomic DNA prepared according to the protocol described above. The microplate method was employed as described by Ezaki *et al.* (1989) and Goris *et al.* (1998), by using an HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. Biotinylated DNA was hybridized with unlabelled single-stranded DNA which was bound non-covalently to microplate wells. Hybridizations were performed at 50 °C in a hybridization mixture (2 × SSC, 5 × Denhardt's solution, 2.5 % dextran sulfate, 50 % formamide, 100 μg denatured salmon sperm DNA ml^{-1}, 1250 ng biotinylated probe DNA ml^{-1}). Levels of DNA–DNA relatedness were calculated as means based on at least two independent hybridization experiments; reciprocal reactions were performed and considered as independent hybridization experiments. Levels of DNA–DNA relatedness of 6–8 % were found between strain LMG 22367^T and the type strains of recognized *Mycetocola* species, suggesting that the Reblochon isolates represent a novel species.

Peptidoglycan analyses were carried out to establish whether strains LMG 22367^T, LMG 23020, LMG 23021 and LMG 23022 represented a novel *Mycetocola* species or a new genus in the family *Microbacteriaceae*. Towards this end, the three isolates and the type strains of the three recognized *Mycetocola* species were grown in brain heart infusion broth (Difco) for 5 days at 28 °C, and the resultant biomass was washed twice in distilled water and freeze-dried. Purified peptidoglycan preparations were obtained after disruption of cells by shaking with glass beads and subsequent trypsin digestion, according to the method of Schleifer (1985). The amino acids and peptides in the cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates by using the solvent systems described by Schleifer (1985). The N-terminal amino acids of the interpeptide bridges were detected by dinitrophenylation, as described by Schleifer (1985). The molar ratios of the amino acids were determined by GC and GC-MS of *N*-heptafluorobutyryl amino acid isobutyl esters (MacKenzie, 1987; Groth *et al.*, 1996). A standard procedure was also used to determine the muramic acid type (Uchida *et al.*, 1999).

Cell-wall hydrolysates of the novel strains contained major amounts of lysine as the diagnostic cell-wall diamino acid; the molar ratios of lysine, alanine, glycine and glutamic acid were estimated to be 1.0 : 0.7 : 1.1 : 1.8 for strain LMG 23020, 1.0 : 0.6 : 1.1 : 1.6 for strain LMG 22367^T, 1.0 : 6.8 : 1.6 : 2.0 for strain LMG 23021, 1.0 : 0.6 : 1.0 : 1.6 for *Mycetocola lacteus* DSM 15177^T, 1.0 : 0.5 : 0.8 : 1.7 for *Mycetocola saprophilus* DSM 15178^T and 1.0 : 0.7 : 1.0 : 1.7 for *Mycetocola tolaasinivorans* DSM 15179^T; the unusually high alanine content found for strain LMG 23021 might be due to contaminating protein. The peptidoglycan preparations of the *Mycetocola* type strains, like those of the new isolates (except strain LMG 23021), had a low alanine content, and more than one glutamic acid residue per lysine residue. Alanine occurred as the N-terminal amino acid of the interpeptide bridge in *Mycetocola saprophilus* DSM 15178^T, *Mycetocola tolaasinivorans* DSM 15179^T and strains LMG 22367^T, LMG 23020 and LMG 23021. The peptide patterns of the partially hydrolysed peptidoglycan of all of the novel strains were very similar, consisting of the dipeptides L-Ala–D-Glu, Gly–D-Glu and L-Lys–D-Ala; these data justify the conclusion that these strains have a *β*-type peptidoglycan

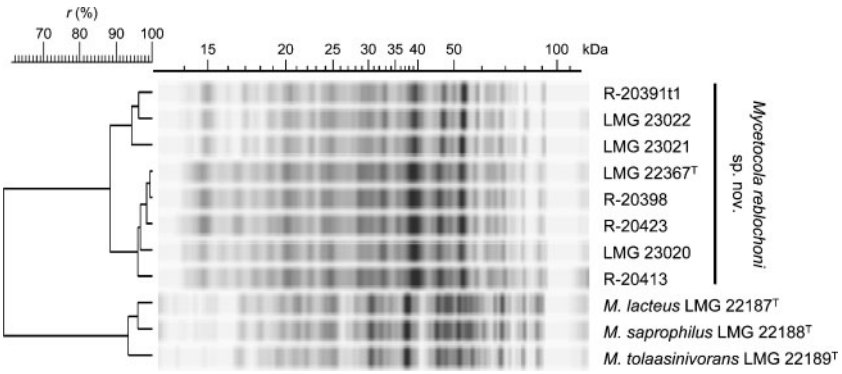

Fig. 2. SDS-PAGE protein patterns of the new isolates and the type strains of recognized *Mycetocola* species. The dendrogram was generated by UPGMA linkage of correlation coefficients (*r*, expressed for convenience as percentage similarity).

(Schleifer, 1985), a finding that is in line with the phylogenetic positions of the organisms. It is evident from these results that the peptidoglycan structure of the new isolates and of the type strains of the *Mycetocola* strains are very similar. Indeed, the peptidoglycan data differ from those of all known peptidoglycans based on lysine (http://www.dsmz.de/microorganisms/main.php?content_id=35). All of the new isolates contained *N*-acetylated muramic acid.

Strain LMG 22367^T and the type strains of the *Mycetocola* species were examined for additional chemotaxonomic markers. Polar lipids and isoprenoid quinones were extracted from freeze-dried biomass following the procedure described by Minnikin *et al.* (1984). The polar lipids were separated by using a standard TLC technique (Minnikin *et al.*, 1984) and the isoprenoid quinone extracts were filtered and reduced to dryness in a stream of nitrogen. The resultant dried preparations were dissolved in 200 µl propanol and filtered by using a Dyna Gard 0.2-µl syringe filter and 5 µl of the filtered extract was separated by HPLC following Kroppenstedt (1985). Fatty acid methyl esters were prepared and analysed following Klatte *et al.* (1994) by using the standard Microbial Identification System (MIDI system; http://www.midi-inc.com/).

All of the organisms gave a simple polar lipid pattern that consisted of two phospholipids, a major diphosphatidylglycerol spot, a faint phosphatidylglycerol spot and a prominent glycolipid that was visualized by spraying with anisaldehyde. All of the strains contained unsaturated menaquinones with ten isoprene units as the predominant isoprenologue, although strain LMG 22367^T produced smaller proportions of MK-9 and MK-11 compared with the other strains and lacked MK-8 (Table 1). It can be seen from this table that the strains also shared similar fatty acid profiles, with 12-methyl tetradecanoic acid as the major component, although all four strains could be separated based on quantitative differences in major fatty acid components.

The presence of lysine in the cell-wall peptidoglycan of the new isolates readily distinguished them from genera of the family *Microbacteriaceae* that contain other cell-wall diamino acids (Zhang *et al.*, 2007). Additional chemical markers could be used to distinguish the new isolates from genera in the family that contain lysine in the peptidoglycan. Thus, strain

Table 1. Fatty acid and menaquinone profiles (%) of strain LMG 22367^T and the type strains of recognized *Mycetocola* species

Strains: 1, LMG 22367^T; 2, *Mycetocola lacteus* DSM 15177^T; 3, *Mycetocola saprophilus* DSM 15178^T; 4, *Mycetocola tolaasinivorans* DSM 15179^T. −, Not detected. Abbreviations are exemplified by the following: anteiso-15 : 0, 12-methyl tetradecanoic acid; 16 : 0, hexadecanoic acid; iso-16 : 0, 14-methyl pentadecanoic acid.

Component	1	2	3	4
Fatty acids				
anteiso-13 : 0	−	−	−	0.09
iso-14 : 0	0.42	0.56	0.59	0.83
14 : 0	−	0.81	0.85	2.14
anteiso-15 : 1	0.92	−	−	−
iso-15	0.74	1.90	2.03	0.77
anteiso-15 : 0	44.48	47.88	48.71	50.31
15 : 0	−	1.02	1.09	2.07
iso-16 : 0	10.23	11.2	11.18	9.73
16 : 0	14.26	17.23	17.65	20.31
iso-17 : 0	0.51	0.95	0.90	0.22
anteiso-17 : 0	28.44	17.61	16.29	12.76
17 : 0	−	0.48	0.46	0.52
18 : 0	−	0.31	0.25	0.25
Menaquinones				
MK-8	−	7.4	7.2	9.8
MK-9	2.0	30.7	30.4	30.9
MK-10	95.0	53.2	53.3	50.8
MK-11	3.0	8.7	9.1	8.5

LMG 22367^T could be distinguished from *Frigoribacterium* and *Microcella* strains based on its major fatty acid and menaquinone components (Kämpfer *et al.*, 2000; Tiago *et al.*, 2005), and strains LMG 22367^T, LMG 23020 and LMG 23022 could be distinguished from members of the genera *Microbacterium* and *Okibacterium* as the former contain *N*-acetylated muramic acid and the latter *N*-glycolated muramic acid (Takeuchi & Hatano, 1998; Evtushenko *et al.*, 2002). By contrast, strain LMG 22367^T and the type strains of *Mycetocola lacteus*, *Mycetocola saprophilus* and *Mycetocola tolaasinivorans* showed very similar chemotaxonomic profiles based on the present as well as previous data (Tsukamoto *et al.*, 2001).

Table 2. Differential characteristics between strain LMG 22367^T and the type strains of recognized *Mycetocola* species

Strains: 1, LMG 22367^T (identical results were obtained for strains LMG 23020, LMG 23021 and LMG 23022); 2, *Mycetocola lacteus* LMG 22187^T; 3, *Mycetocola saprophilus* LMG 22188^T; 4, *Mycetocola tolaasinivorans* LMG 22189^T. All strains were positive for aesculin and arbutin hydrolysis, produced acid from cellobiose, fructose, glucose, glycerol, maltose, mannitol and ribose, utilized adonitol, amygdalin, L-arabinose, D-arabinose, arbutin, cellobiose, dextrin, dulcitol, i-erythritol, ethanol, D-fucose, L-fucose, fructose, galactose, glucose, glycerol, glycogen, *myo*-inositol, inulin, lactose, maltotriose, mannitol, mannose, melezitose, melibiose, methyl α-D-glucoside, raffinose, α-L-rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, trehalose, turanose, xylitol and xylose as sole carbon sources, degraded tributyrin and Tween 40, utilized butan-1,3-diol, butan-1,4-diol, butan-1-ol, butan-2,3-diol, isoamyl alcohol and propan-1,2-diol (all at 1 %, v/v), sodium acetate, adipate, butyrate, citrate, fumarate, gluconate, lactate, mandelate, malate, pyruvate, propionate, sebacate, succinate and valerate (at 0.1 %, w/v) as sole carbon sources and L-aspartic acid, L-ornithine and thymidine as sole carbon and nitrogen sources and produced esterase lipase (C8) and naphthol-AS-B1 phosphohydrolase. None of the strains degraded elastin, gelatin, guanine, hypoxanthine, uric acid, xanthine or xylan, hydrolysed allantoin, utilized *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, hydroxybutyric acid, malonate, oleate or oxalate as sole carbon sources or acetamide, L-alanine, L-aminobutyric acid, L-arginine, L-cysteine, L-glutamic acid, L-glycine, L-isoleucine, L-norleucine, DL-methionine, monoethanolamine, DL-norleucine, L-norvaline, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, urea, uric acid or L-valine as sole carbon and nitrogen sources, grew at 37 °C or in the presence of 10 % (w/v) NaCl or produced alkaline phosphatase, *N*-acetyl-β-glucosaminidase, α-fucosidase, β-glucuronidase, lipase (C14) or α-mannosidase.

Characteristic	1	2	3	4
Acid production from:				
Dextrin	−	+	+	−
Galactose	−	+	+	+
Lactose	−	+	+	−
Mannose	+	−	+	+
Raffinose	−	+	+	−
Salicin	+	+	+	−
Sucrose	+	−	+	+
Trehalose	+	+	+	−
Xylose	−	+	+	+
API ZYM tests				
Acid phosphatase	−	+	−	+
α-Chymotrypsin	−	−	+	+
Cystine arylamidase	−	−	+	+
α-Galactosidase	−	+	−	+
β-Galactosidase	−	+	+	+
α-Glucosidase	+	−	+	+
β-Glucosidase	+	−	+	+
Leucine arylamidase	+	−	+	+
Trypsin	−	−	+	+
Valine arylamidase	−	−	+	+
Degradation of:				
Casein	−	+	−	−
DNA	+	−	−	−
Tween 60	−	−	+	+
Growth in the presence of 5 % (w/v) NaCl	+	−	+	+
Growth at pH 10.0	+	−	+	+
Hydrolysis of urea	+	−	+	+
Reduction of nitrate	−	+	+	−
Utilization of sole carbon and nitrogen sources				
L-Asparagine	+	−	−	−
L-Histidine	−	−	+	+

The colonial and cellular morphology of the four representative isolates was examined, as was their ability to grow on PY agar (Tsukamoto *et al.*, 2001) over ranges of temperature and pH. Cellular morphology was examined by electron microscopy following growth on PY agar for 2 days at 30 °C and negative staining with phosphotungstic acid, as described by Tsukamoto *et al.* (2001). Cells were Gram-positive, non-sporulating, non-motile rods that grew well under aerobic conditions. They formed smooth, circular, convex colonies on brain heart infusion agar (Difco). Growth was observed between 20 and 30 °C, but not at 37 °C, and from pH 7.0 to 10, but not at pH 12. All of these properties are consistent with the assignment of the strains to the genus *Mycetocola* (Tsukamoto *et al.*, 2001).

The four representative isolates and the type strains of the three recognized *Mycetocola* species were examined for a range of biochemical and physiological properties as outlined by Kim *et al.* (1998). In addition, API ZYM tests (bioMérieux) were performed following the manufacturer's instructions. It is clear from the data in Table 2 that the new isolates share many phenotypic properties, some of which allow them to be distinguished from the *Mycetocola* type strains.

It can be concluded from the genotypic and phenotypic data presented that the Reblochon cheese isolates represent a novel species of the genus *Mycetocola*, for which the name *Mycetocola reblochoni* sp. nov. is proposed.

Description of *Mycetocola reblochoni* sp. nov.

Mycetocola reblochoni (re.blo.cho′ni. N.L. neut. n. *reblochonum* Reblochon cheese; N.L. gen. n. *reblochoni* of a Reblochon cheese, to denote that the first strains were isolated from the surface of Reblochon cheese).

Aerobic, Gram-positive, asporogenous, non-motile, catalase-positive, rod-shaped actinomycetes (0.3 × 1.1–1.5 μm) that form circular, convex, smooth, shiny colonies on PY agar. Growth occurs between 20 and 30 °C, but not at 37 °C, and between pH 7.0 and pH 10, but not at pH 12.0. Does not hydrolyse allantoin. Additional phenotypic properties are given in Table 2. The organism has

chemotaxonomic properties consistent with its classification in the genus *Mycetocola* and forms a distinct phyletic line in the *Mycetocola* 16S rRNA gene tree. The G+C content of the DNA of the type strain is 70.0 mol%.

The type strain, LMG 22367^T (=R-20377^T =BRB-1L41^T =DSM 18580^T), was isolated from the surface of Reblochon cheese at the late stage of ripening. Reference strains LMG 23020 (=BRB-4LJ1 =R-20402), LMG 23021 (=BRB-4LB3 =R-203813) and LMG 23022 (=BRB-3LJ5 =R-203891) were isolated from the same source.

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