Microbacterium hatanonis sp. nov., isolated as a contaminant of hairspray

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An aerobic, rod-shaped, Gram-positive, oxidase-negative, catalase-positive bacterial isolate, strain FCC-01^T, originating as a contaminant of hairspray was characterized using phenotypic and molecular taxonomic methods. A 16S rRNA gene sequence analysis revealed that the isolate belonged to the genus *Microbacterium* and represented an evolutionary lineage that was distinct from recognized *Microbacterium* species. Cell-wall hydrolysate from the isolate contained ornithine and the cell-wall sugars consisted of rhamnose and galactose. The main respiratory quinones were MK-12 (38 %) and MK-11 (35 %). The major cellular fatty acids were anteiso-C_{15:0} (48 %), anteiso-C_{17:0} (35 %) and iso-C_{16:0} (11 %). The DNA G + C content was 69 mol%. The isolate showed <98 % 16S rRNA gene sequence similarity with respect to all of the *Microbacterium* species with validly published names. On the basis of the morphological, physiological and chemotaxonomic data and the results of the comparative 16S rRNA gene sequence analysis, this isolate represents a novel species of the genus *Microbacterium*, for which the name *Microbacterium hatanonis* sp. nov. is proposed. The type strain is FCC-01^T (=JCM 14558^T =DSM 19179^T).

The genus *Microbacterium* was described by Orla-Jensen (1919) and the type strain of *Microbacterium lacticum* (ATCC 8180^T) was isolated during studies on lactic acid-producing bacteria. Collins *et al.* (1983) and Takeuchi & Hatano (1998) amended the description of the genus *Microbacterium*. At the time of writing, the genus *Microbacterium* comprises 46 species with validly published names, including the recently reported *Microbacterium terricola* (Kageyama *et al.*, 2007a, b). The *Microbacterium* species described in the literature have been isolated from milk products, various environmental sources, including soil, water and plants, and from clinical samples, e.g. human blood cultures (*Microbacterium resistens* and *Microbacterium paraoxidans*; Funke *et al.*, 1998; Behrendt *et al.*, 2001; Laffineur *et al.*, 2003) and catheter-related bacteraemia samples and bone-marrow products (Funke *et al.*, 1995; Lau *et al.*, 2002; Hirji *et al.*, 2003). Contaminated cosmetic products are relatively uncommon, but certain products can be contaminated with micro-organisms and are unable to suppress the growth of several micro-organisms, representing a potential health hazard. Cosmetic products that were contaminated by bacteria during consumer use have been described by

Brannan & Dille (1990) and Campana *et al.* (2006). However, we are unaware of any published information concerned with the occurrence of *Microbacterium* species in cosmetic products.

A yellow-pigmented bacterium, designated strain FCC-01^T, was isolated on trypticase soy agar (TSA) medium at 30 °C during the course of studies designed to identify microbial contaminants of hairspray. The isolate and reference strains *Microbacterium aurum* JCM 9179^T, *M. lacticum* JCM 1379^T and *Microbacterium schleiferi* JCM 9175^T were cultured on TSA for 72 h at 30 °C. *Microbacterium terregens* JCM 1342^T was cultured on brain-heart infusion agar with soil extract (BHIASE) (JCM medium no. 342; http://www.jcm.riken.jp/cgi-bin/jcm/jcm_grmd?GRMD=342) at 30 °C for 72 h. The cell morphology was examined by using light microscopy. For motility testing, the isolate was stab-inoculated into tubes containing semi-solid TSA and BHIASE (0.5 %) and incubated at 30 °C for up to 72 h (McClung & Lindberg, 1957).

Growth at different temperatures (4, 10, 20, 30, 37, 41 and 50 °C) was tested on TSA. Anaerobic growth in a 100 % CO₂ atmosphere and microaerophily (candle-jar method; Gerhardt *et al.*, 1981) were examined after incubation of the novel strain on the same medium at 30 °C for 1 week in anaerobic jars. The ability to grow at various NaCl concentrations (1, 2, 5, 10 and 15 %, w/v) and pH values (5.0, 6.0, 7.0, 8.0, 9.0 and 10.0; adjusted with 6 M HCl or 3 M NaOH) was examined at 30 °C using TSA as the basal

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FCC-01^T is AB274908.

Neighbour-joining and minimum-evolution phylogenetic trees and cellular fatty acid compositions for isolate FCC-01^T in relation to *Microbacterium* species are available as supplementary material with the online version of this paper.

medium. Oxidase activity was tested using tetramethyl-*p*-phenylenediamine and catalase activity was tested using 3 % H₂O₂. Commercially available API 20NE and API ZYM systems were used, according to the instructions of the manufacturer (bioMérieux), to determine the biochemical characteristics.

Cellular fatty acids were analysed by growing cells on TSA and BHIASE for 48 h at 30 °C. Saponification, methylation and extraction of cellular fatty acids and determination of cellular fatty acid profiles were performed according to the instructions provided with the Sherlock Microbial Identification System (MIDI) as described previously by Sakamoto *et al.* (2002). Menaquinones were extracted and analysed according to Sakamoto *et al.* (2002), whole-cell sugars were analysed by using HPLC as described previously (Mikami & Ishida, 1983) and the amino acid composition was determined according to the method described by Komagata & Suzuki (1987).

DNA was extracted and purified by following the method described by Marmur (1961). Two universal primers (27F, 5'-AGAGTTTGTATCCTGGCTCAG-3'; 1492R, 5'-GGTTCCTTGTTACGACTT-3') were used for PCR amplification of the 16S rRNA gene. The amplified PCR product was purified using a Multiscreen-PCR₉₆ filter plate (Millipore) and then sequenced using an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) with an automatic DNA sequencer (model 3100; Applied Biosystems/Hitachi).

An almost-complete 16S rRNA gene sequence (1484 bp) was determined for isolate FCC-01^T and aligned with the 16S rRNA gene sequences of representatives of the genus *Microbacterium* by using CLUSTAL_X software (version 1.81) (Thompson *et al.*, 1997). Parsimony analysis was carried out with the maximum-parsimony method implemented in the PAUP (version 4.0b10) software package (Swofford, 2000). A neighbour-joining (Saitou & Nei, 1987) phylogenetic tree was inferred using the software package MEGA, version 3.1 (Kumar *et al.*, 2004). Neighbour-joining phylogenetic trees were constructed according to the Kimura-2 model (Kimura, 1980). A minimum-evolution (Rzhetsky & Nei, 1992) phylogenetic tree was inferred using the same software package. The topologies of the phylogenetic trees were evaluated using bootstrap analysis (Felsenstein, 1985) based on 1000 replications.

The DNA G+C content was determined using HPLC (Tamaoka & Komagata, 1984) after enzyme digestion of the DNA to deoxyribonucleosides. An equimolar mixture of four deoxyribonucleotides from a Yamasa GC kit (Yamasa Shoyu) was used as the quantitative standard.

Cells of strain FCC-01^T were aerobic, rod-shaped and Gram-positive. Motility was not observed under the growth conditions used. Colonies were circular, convex with entire margins, moist, shiny and light yellow in colour. The isolate demonstrated microaerophilic growth, but was not able to grow under anaerobic conditions. The

isolate grew at 10–37 °C, at a maximum NaCl concentration of 2 % and at pH 6.0–9.0. Optimum growth was observed at 30 °C without the addition of NaCl.

The phylogenetic analysis showed that strain FCC-01^T belongs to the genus *Microbacterium* (Fig. 1; also see Supplementary Figs S1 and S2 available in IJSEM Online), sharing the highest 16S rRNA gene sequence similarity with respect to *M. schleiferi* NBRC 15075^T (97.8 %). The sequence similarities with respect to all of the other *Microbacterium* species with validly published names were below 98 %, the value recognized by the International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of the suborder *Micrococccineae* (Schumann & Evtushenko, 2006) to be sufficient for species delineation at the genotypic level.

The morphological, physiological and chemotaxonomic characteristics of strain FCC-01^T (shown in Table 1 and the species description) were typical of members of the genus *Microbacterium* (Takeuchi & Hatano, 1998; Behrendt *et al.*, 2001): the peptidoglycan diamino acid of the strain was ornithine, the major menaquinones were MK-12 (37.5 %), MK-11 (34.8 %) and MK 10 (11.6 %) and the predominant fatty acids were anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0} (Supplementary Table S1). However, strain FCC-01^T clearly differed from phylogenetically related species (Table 1): for instance, in contrast to *M. schleiferi*, it utilized arabinose as a sole carbon source, produced acid phosphatase, α-galactosidase, β-glucuronidase and α-fucosidase and failed to grow at 41 °C. The cell-wall sugars of FCC-01^T were rhamnose and galactose, whereas *M. schleiferi* contained 6-deoxytalose.

The DNA G+C contents of the isolate, *M. schleiferi* JCM 9175^T, *M. lacticum* JCM 1379^T, *M. aurum* JCM 9179^T and

Fig. 1. Maximum-parsimony phylogenetic tree, constructed from a comparative analysis of 16S rRNA gene sequences, showing the relationships of isolate FCC-01^T with respect to type strains of related *Microbacterium* species. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. *Arthrobacter globiformis* DSM 20124^T served as the outgroup. Bar, 10 substitutions per site. Neighbour-joining and minimum-evolution phylogenetic trees are given in Supplementary Figs S1 and S2.

Table 1. Characteristics that differentiate isolate FCC-01^T from phylogenetically related *Microbacterium* species

Strains/species: 1, strain FCC-01^T; 2, *M. schleiferi* JCM 9175^T; 3, *M. terricola*; 4, *M. keratanolyticum*; 5, *M. aoyamense*; 6, *M. terregens* JCM 1342^T; 7, *M. aurum* JCM 9179^T; 8, *M. lacticum* JCM 1379^T; 9, *M. koreense*; 10, *M. oleivorans*; 11, *M. hydrocarbonoxydans*; 12, *M. resistens*. Data for the type strains of *M. schleiferi*, *M. aurum*, *M. lacticum* (API 20NE and API ZYM tests) and *M. terregens* (API ZYM test) are from this study; other data for these species and data for other reference taxa were taken from Yokota *et al.* (1993), Funke *et al.* (1998), Behrendt *et al.* (2001), Schippers *et al.* (2005), Kageyama *et al.* (2006), Lee *et al.* (2006) and Kageyama *et al.* (2007a). +, Positive; –, negative; v, variable; w, weak; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Colony colour*	LY	Y	Y	Y	LY	Y	Y	Y	LY	O	Y	Y
Temperature for growth (°C)	10–37	10–42	10–35	28	14–34	20–30	10–42	15–30	20–37	30–37	30–37	37
Growth in 5% NaCl	–	–	–	–	+	–	+	–	+	–	–	ND
DNA G+C content (mol%)	69.0	66.5	70.0	66.5	69.0	68.4	69.0	68.8	68.0	ND	ND	ND
Major menaquinones	12, 11	10, 11, 12	12, 13	12, 13	12, 13, 14	12, 13	11, 12	11, 12	11, 12	11, 12	11, 12	ND
Cell-wall diamino acid†	Orn	Orn	Orn	Orn	Orn	Orn	Lys	Lys	Lys	Lys	Orn	Orn
Cell-wall sugar(s)‡	Rha, Gal	6dTal	ND	Gal	Rha, Gal, Xyl	Rha, Gal, 6dTal	Fuc, Gal, Glc	Rha, Man, Gal	Gal, Xyl	ND	ND	ND
Nitrate reduction	–	–	ND	+	ND	+	–	+	–	ND	ND	–
Assimilation of:												
Glucose	+	w	+	ND	+	–	+	+	+	+	+	ND
L-Arabinose	+	–	ND	+	–	–	–	–	–	+	+	–
D-Mannose	+	+	+	ND	+	–	w	+	+	+	+	+
D-Mannitol	+	+	ND	ND	+	–	+	+	+	+	+	ND
N-Acetylglucosamine	–	–	ND	+	+	–	+	+	+	–	+	+
Maltose	+	+	+	ND	+	–	+	+	+	+	+	ND
Potassium gluconate	+	+	ND	ND	ND	–	+	+	ND	+	+	+
Malate	w	–	ND	–	ND	+	–	+	ND	+	+	–
Trisodium citrate	–	–	ND	–	ND	–	–	+	ND	–	+	+
Phenylacetic acid	–	–	ND	–	ND	–	–	–	ND	–	+	–
Enzyme activities												
Alkaline phosphatase	–	–	+	ND	–	+	–	–	ND	ND	ND	ND
Lipase	–	–	w	ND	+	–	–	–	ND	ND	ND	ND
Cystine arylamidase	+	+	+	ND	–	+	+	+	ND	ND	ND	ND
Trypsin	+	w	+	ND	–	+	+	w	ND	ND	ND	ND
Acid phosphatase	+	–	+	ND	+	+	+	+	ND	ND	ND	+
α-Galactosidase	+	–	v	ND	–	–	+	–	ND	ND	ND	–
β-Galactosidase	+	+	ND	ND	+	–	+	+	–	+	+	ND
β-Glucuronidase	+	–	–	ND	–	–	–	–	ND	–	–	–
α-Glucosidase	+	+	+	ND	+	+	w	–	ND	+	+	+
β-Glucosidase	+	+	+	ND	+	w	–	–	ND	+	+	+
α-Mannosidase	–	–	–	ND	–	–	+	–	ND	+	+	+
α-Fucosidase	+	–	–	ND	–	–	–	–	ND	ND	ND	+

*LY, Light yellow; O, orange; Y, yellow.

†Lys, Lysine; Orn, ornithine.

‡6dTal, 6-Deoxytalose; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Xyl, xylose.

M. terregens JCM 1342^T were 69, 67, 69, 69 and 68 mol%, respectively. The DNA G+C content of the isolate corresponds with those of the members of the genus *Microbacterium*, which have high G+C contents (66.5–71.6 mol%; Takeuchi & Hatano, 1998).

Taken together, the data presented here indicate that strain FCC-01^T represents a novel species within the genus *Microbacterium*, for which the name *Microbacterium hatanonis* sp. nov. is proposed.

Description of *Microbacterium hatanonis* sp. nov.

Microbacterium hatanonis (ha.ta.no'nis. N.L. gen. masc. n. *hatanonis* of Hatano, in honour of Dr Kazunori Hatano, for his contribution to the understanding of the genus *Microbacterium*).

Aerobic, non-motile, Gram-positive organism. Microaerophilic; does not grow on TSA under an anaerobic atmosphere (CO₂). Typically, cells are rods, approximately

0.8 µm wide and 1.7 µm long. Colonies are circular, convex with entire margins, moist, shiny and light yellow. The temperature range for growth is 10–37 °C, with optimal growth at 30 °C. The pH range for growth is 6.0–9.0, with optimal growth at pH 7.0. No growth occurs at NaCl concentrations above 2%; optimal growth occurs without the addition of NaCl. Demonstrates positive results for catalase, aesculin hydrolysis, β-galactosidase and assimilation of glucose, arabinose, mannose, mannitol, maltose and potassium gluconate. Weakly positive for malate assimilation. Gives positive results for the production of esterase, esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and α-fucosidase. Shows negative results for oxidase, nitrate reduction, indole production, D-glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, utilization of N-acetylglucosamine, capric acid, adipic acid, trisodium citrate and phenylacetic acid and for the production of alkaline phosphatase, lipase, α-chymotrypsin and α-mannosidase. The DNA G + C content is 69.0 mol%, the major isoprenoid quinones are MK-12 (37.5 %) and MK-11 (34.8 %) and the major cellular fatty acids are anteiso-C_{15:0} (48.1 %), anteiso-C_{17:0} (34.7 %) and iso-C_{16:0} (10.5 %). The cell-wall sugars are rhamnose and galactose. Diamino acid ornithine is present in the cell-wall hydrolysates.

The type strain, FCC-01^T (=JCM 14558^T =DSM 19179^T), was isolated from hairspray.

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