Helcobacillus massiliensis gen. nov., sp. nov., a novel representative of the family *Dermabacteraceae* isolated from a patient with a cutaneous discharge

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Gram-positive, non-spore-forming rods (strain 6401990^T), isolated from a human cutaneous discharge were subjected to a polyphasic taxonomy study. The only respiratory quinone was MK-7 and the major fatty acids were anteiso-C15:0 (34.3%), anteiso-C17:0 (18.7%) and iso-C16:0 (18.6%). Mycolic acids were not present. Polar lipids present were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and unidentified glycolipids. The isomer of diaminopimelic acid identified was *meso*-diaminopimelic acid, and the analysis of whole-cell sugars showed the presence of high amounts of galactose, ribose and some glucose. The G + C content of strain 6401990^T was 68.6%. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed 95.1% similarity with *Dermabacter hominis*. On the basis of phenotypic data and phylogenetic inference, it is proposed that this strain represents a novel species in a new genus of the family *Dermabacteraceae*, for which the name *Helcobacillus massiliensis* gen. nov., sp. nov. is proposed. The type strain is 6401990^T (CSUR P17^T=CIP 109418^T=CCUG 53859^T).

The family *Dermabacteraceae* consists of Gram-positive, non-spore-forming, non-motile, rod-shaped bacteria that have DNA with a high G + C content (Stackebrandt *et al.*, 1997). Presently, it includes the genera *Dermabacter*, *Brachybacterium* and a recently described genus *Devriesea* (Martel *et al.*, 2008). Four strains were studied in 1988 and assigned to a novel genus and species, *Dermabacter hominis* (Jones & Collins, 1988). These strains were isolated from human skin, but there was no evidence for the pathogenicity of the bacteria. In 1994, it was established that CDC group 3 and group 5 coryneform bacteria were members of the genus *Dermabacter* (Funke *et al.*, 1994; Gruner *et al.*, 1994). On the basis of DNA–DNA hybridization values, most of the strains were considered to belong to the species *D. hominis*. However, eight strains showed DNA–DNA hybridization values <70% with *D. hominis*, but they were not included in other species because they could not be phenotypically differentiated. These strains were isolated from blood, establishing *D. hominis* as a potential human pathogen. In recent years, this bacterium was successively described in cerebral abscess (Bavbek *et al.*, 1998), bacteraemias (Gómez-Garcés *et al.*, 2001), peritoneal dialysis-associated peritonitis (Radtke *et al.*, 2001) and chronic osteomyelitis (Van Bosterhaut *et al.*, 2002),

highlighting its role as an opportunistic pathogen. The genus *Brachybacterium* was proposed by Collins *et al.* (1988) and presently includes 12 recognized species. They were isolated from environmental samples (*Brachybacterium faecium*, *Brachybacterium fresconis*, *Brachybacterium sacelli*, *Brachybacterium phenoliresistens* and *Brachybacterium zhongshanense*), cheeses (*Brachybacterium alimentarium* and *Brachybacterium tyrofermentans*), corn steep liquor (*Brachybacterium conglomeratum*, *Brachybacterium paraconglomeratum* and *Brachybacterium rhamnorum*), milk (*Brachybacterium nesterenkovi*) and murine liver (*Brachybacterium muris*). Their implication in human pathology was not demonstrated. The genus *Devriesea* was recently proposed by Martel *et al.* (2008) and includes the species *Devriesea agamarum* associated with dermatitis and septicaemia in agamid lizards.

In this report, we attempted to elucidate the phylogenetic position of strain 6401990^T which was isolated from a human cutaneous discharge sample. Using a polyphasic taxonomic approach, it is proposed that the isolate represents a novel genus of the family *Dermabacteraceae*.

A 58-year-old man was admitted to hospital in September 2006 for asthenia, myalgia and diffuse pain predominantly occurring in rachis, and presenting a chronic evolution over the course of 1 year. Antinuclear antibodies were weakly positive. A pathology such as connectivitis was suspected but could be neither confirmed nor eliminated. During his hospitalization, the patient presented an

Abbreviations: DAP, diaminopimelic acid; *meso*-Dpm, *meso*-diaminopimelic acid.

The GenBank accession number for the 16S rRNA gene sequence of *Helcobacillus massiliensis* strain 6401990^T is EU477050.

erythrasma. The cutaneous discharge sample was tested in our laboratory. The isolate was first identified as *Corynebacterium minutissimum*, using the miniaturized API Coryne strip (bioMérieux), but the phenotypic identification remained unclear. Consequently, genotypic identification was performed by 16S rRNA gene sequence comparison and strain 6401990^T was found to cluster in the *Dermabacteraceae* family close to *Dermabacter hominis*. The patient was empirically treated by oral erythromycin for 7 days. The erythrasma regressed quickly.

MICs were determined according to the disc diffusion method that we currently use in our laboratory, as there are, to our knowledge, no international guidelines for the antimicrobial susceptibility testing of coryneform bacteria. Strain 6401990^T was found to be susceptible to amoxicillin, vancomycin, rifampicin, doxycycline and gentamicin, and intermediate to erythromycin.

Surface colonies on sheep blood agar (bioMérieux) were circular, white, shiny and smooth after 24 h aerobic incubation at 37 °C. Growth and haemolytic activity were tested at 37 °C on Columbia agar with 5 % sheep blood. Growth was tested after 24 h incubation at 37 °C, in the presence of air or 5 % CO₂, and also in anaerobic and microaerophilic atmospheres which were created using GENbag anaer and GENbag microaer (bioMérieux), respectively. Growth at different temperatures (25, 30, 37, 44 and 50 °C) was tested on Columbia agar for 24 h. Optimum growth was obtained at 37 °C, but occurred between 25 and 44 °C. The strain was aerobic and also grew in microaerophilia and in the presence of 5 % CO₂. Growth in an anaerobic atmosphere was weak after 48 h incubation. The bacteria were non-haemolytic after 24 h incubation, but became alpha-haemolytic after 48 h incubation in the presence of air. The size and ultrastructure of cells were determined by transmission electron microscopy. Cells were grown in trypticase soy broth for 24 h at 37 °C. The bacterial suspension was pre-fixed in 5 % (v/v) glutaraldehyde in phosphate buffer (Gibco; KCl, 200 mg l⁻¹; KH₂PO₄, 200 mg l⁻¹; NaCl, 8 g l⁻¹; Na₂HPO₄·7H₂O, 2.16 g l⁻¹) for at least 1 h at room temperature, washed in the same buffer and stained with 1 % (w/v) phosphotungstic acid. The samples were examined on a Morgagni 268D (Philips) electron microscope at an operating voltage of 60 kV. The rods were 0.7–1 µm long and 0.4–0.7 µm wide.

Catalase activity determined by the ID colour Catalase test kit (bioMérieux) was positive. Oxidase activity was assayed by applying the cells to moistened discs that were impregnated with dimethyl-*p*-phenylenediamine (bioMérieux) and was negative.

The commercially available API ZYM, API Coryne and API 50 CH strips combined with API 50CHB/E medium (bioMérieux) were used to characterize the biochemical properties of the strain according to the manufacturer's instructions, and incubation was performed at 37 °C for 4, 24 and 72 h, respectively. Phenotypic characteristics were

compared to those of *D. hominis* CCUG 32998^T which was the closest related species when 16S rRNA gene sequences were compared. Characteristic traits are presented in Table 1. The results of the other tests for the type strain are given in the species description below.

The biochemical characteristics of strain 6401990^T were also determined using the Biolog System. Bacterial growth was obtained on BUG (Biolog Universal Growth) agar + blood (Biolog) for 24 h at 37 °C. The bacterial suspension was prepared in sterile GP/GN inoculation fluid (Biolog) and inoculated into GP2 Microplates (Biolog; to study compounds as sole carbon sources) as described by the manufacturer. Incubation was carried out at 36 °C in an aerobic atmosphere for 21 h. The results of the tests are given in the species description below.

Analysis of respiratory quinones by HPLC and polar lipids by two-dimensional TLC were carried out by the Identification Service and Dr Brian Tindall, DSMZ, Braunschweig, Germany. Respiratory lipoquinones were extracted from 100 mg freeze-dried cell material as described by Tindall (1990a, b). Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel, using hexane/*tert*-butylmethylether (9:1, v/v) as solvent. UV absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC at 269 nm. The only respiratory quinone was MK-7. Polar lipids were extracted from 100 mg freeze-dried cell material using a chloroform/methanol/0.3 % aqueous NaCl mixture (1:2:0.8, by vol.; modified after Bligh & Dyer, 1959). The extraction solvent was stirred overnight and the cell debris pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform/methanol/0.3 % aqueous NaCl mixture to a ratio of 1:1:0.9 (by vol.). Polar lipids were separated as described by Tindall *et al.* (2007). The polar lipids present were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and unidentified glycolipids. Preparation and determination of cellular fatty acids were carried out by following the procedures given for the Sherlock Microbial identification System (MIDI). The major fatty acids were anteiso-C15:0 (34.3 %), anteiso-C17:0 (18.7 %) and iso-C16:0 (18.6 %). Mycolic acids were determined as described by Klatte *et al.* (1994) and were not detected. The DNA base composition was determined by using the HPLC method of Mesbah *et al.* (1989). The value for strain 6401990^T was 68.6 mol%.

Whole cells of strain 6401990^T were hydrolysed (4 M HCl, 100 °C, 16 h) and the hydrolysate was subjected to TLC on cellulose plates using the solvent system of Rhuland *et al.* (1955). The isomer of diaminopimelic acid (DAP) identified was *meso*-diaminopimelic acid (*meso*-Dpm). For analysis of whole-cell sugars, cells were hydrolysed in 0.5 M H₂SO₄ (2 h at 100 °C). H₂SO₄ was removed according to the method of Whifton *et al.* (1985) and the

Table 1. Diagnostic traits differentiating *H. massiliensis* 6401990^T and *D. hominis* CIP105144^T

+, Positive; –, negative; w, weak positive; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PL, unidentified phospholipids; GL, unidentified glycolipid; PE, phosphatidylethanolamine; PC, phosphatidylcholine.

Character	<i>D. hominis</i>		<i>H. massiliensis</i>	
	48 h	72 h	48 h	72 h
Acid production from (API 50CH):				
Glycerol	–	–	–	w
Erythritol	–	–	–	+
L-Arabinose	–	–	+	+
D-Ribose	+	+	–	–
D-Xylose	–	–	+	+
D-Galactose	+	+	–	–
Inositol	–	–	+	+
D-Mannitol	–	–	+	+
Methyl- α -D-glucopyranoside	+	+	–	–
Amygdalin	w	+	–	–
Aesculin	+	+	–	–
D-Lactose	+	+	–	–
Melibiose	+	+	–	–
Raffinose	+	+	–	–
Gentiobiose	w	+	–	–
Enzyme assay (API ZYM):				
Esterase lipase (C8)	+		–	
Leucine arylamidase	+		–	
β -Galactosidase	+		–	
β -Glucosidase	+		–	
α -Mannosidase	+		–	
API Coryne strip:				
Reduction of nitrate	–		+	
Pyrazinamidase	–		+	
Pyrrolidonyl arylamidase	+		–	
Alkaline phosphatase	w		–	
Gelatin hydrolysis	–		+	
Xylose fermentation	–		+	
Mannitol fermentation	–		+	
Ribose fermentation	+		–	
Predominant respiratory quinone	MK-9		MK-7	
G + C content (mol%)	62		68.6	
Polar lipids	DPG, PG, PL, GL		DPG, PG, PE, PC, GL	
Fatty acids (%):				
anteiso-C17:0	40–60		18.7	
anteiso-C15:0	13–35		34.3	

sugars in the hydrolysate were analysed by TLC on cellulose plates according to the method of Staneck & Roberts (1974). High amounts of galactose, ribose and some glucose were found.

Bacterial DNA was extracted using the MagNA Pure LC DNA isolation kit III (Roche) with the MagNA Pure LC instrument, as described by the manufacturer.

PCR amplification of the 16S rRNA gene was performed using the universal primer pair fD1 and rp2 (Weisburg *et al.*, 1991). PCR products were purified using MultiScreen PCR (Millipore) and sequencing reactions were carried out using a DNA sequencing kit (BigDye Terminator Cycle

Sequencing v1.1 Ready Reactions; PE biosystems), according to the manufacturers' instructions. Sequencing products were purified and electrophoresis was performed with the 3130 Genetic Analyzer (Applied Biosystems). The obtained sequences were compared with sequences deposited in the GenBank database by using the BLAST program through the NCBI server. Gene sequences were aligned using the multisequence alignment program CLUSTAL_X 1.8 (Thompson *et al.*, 1997). Phylogenetic relationships with closely related species were determined by using MEGA version 4.0 (Tamura *et al.*, 2007). Distance matrices were determined following the assumptions described by Kimura (1980) and were used to elaborate a dendrogram

using the neighbour-joining method (Saitou & Nei, 1987). The maximum-parsimony algorithm was also used to infer phylogenetic relationships. A bootstrap analysis (bootstrap values were obtained for a consensus tree based on 100 randomly generated trees) was performed to investigate the stability of the trees obtained. The clustering of the new isolate was the same with the two methods. The phylogenetic analysis demonstrated that our isolate clustered with *D. hominis* (Fig. 1). The percentage similarity between 16S rRNA gene sequences was determined by using LALIGN (www.ch.embnet.org/software/LALIGN_form.html). The value between strain 6401990^T and *D. hominis* was 95.1 %. This value is <97 % (which is the gold standard value to determine the species level) (Stackebrandt *et al.*, 2002), confirming that strain 6401990^T belongs to a novel species, but the different phenotypic characteristics implied that strain 6401990^T did not belong to the genus *Dermabacter*.

Based on the results described above, we propose the name *Helcobacillus massiliensis* gen. nov., sp. nov. for the described bacterium. The type strain is 6401990^T.

Description of *Helcobacillus* gen. nov.

Helcobacillus (Hel.co.ba.cil'lus. Gr. n. *helkos*, wound; L. masc. n. *bacillus*, rod; N.L. masc. n. *Helcobacillus*, a rod found in wounds).

Gram-positive, non-spore-forming, non-motile, short rods. Essentially aerobic but may show very weak growth under anaerobic conditions. Optimum growth is obtained at 37 °C. Catalase-positive and oxidase-negative. Acid is produced from glucose and some other sugars. Nitrate is reduced. Starch and aesculin are not hydrolysed. Gelatin is liquefied. Activities of *N*-acetyl- β -glucosaminidase, pyrazinamidase and α -glucosidase are detected. The only menaquinone is MK-7. Predominant cellular fatty acids are anteiso-C15:0 (34.3 %), anteiso-C17:0 (18.7 %) and iso-C16:0 (18.6 %). Mycolic acids are absent. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and unidentified glycolipids (phospholipid pattern III). The isomer of

DAP is *meso*-Dpm. Analysis of whole-cell sugars reveals high amounts of galactose, ribose and some glucose. The G+C content of the genomic DNA of the type species is 68.6 mol%. The type species is *Helcobacillus massiliensis*.

Description of *Helcobacillus massiliensis* sp. nov.

Helcobacillus massiliensis (mas.si.li.en'sis. L. masc. adj. *massiliensis* of Massilia, the old Roman name for Marseille, where the type strain was isolated).

Cells are aerobic, Gram-positive, short, irregular, straight, non-spore-forming, and non-motile rods. Catalase-positive and oxidase-negative. Essentially aerobic but may show very weak growth under anaerobic conditions. Optimal growth occurs at 37 °C. After 24 h growth on sheep blood agar, surface colonies are up to 1 mm diameter, smooth, circular, white and shiny. The rods measure 0.7–1 μ m in length and 0.4–0.7 μ m in diameter (as determined by electron microscopy). Using API ZYM, activities of *N*-acetyl- β -glucosaminidase and α -glucosidase are detected. Activities of leucine arylaminidase, valine arylaminidase, β -galactosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylaminidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase are not detected. Using API 50CH strips after an incubation time of 48 h, acid is produced from L-arabinose, D-xylose, D-glucose, D-fructose, inositol, D-mannitol, maltose, sucrose and turanose. After an incubation time of 72 h, acid is produced (besides the characters previously quoted) from glycerol (weakly), erythritol, *N*-acetylglucosamine (weakly), trehalose (weakly) and melezitose (weakly). Acid is not produced from D-arabinose, D-ribose, L-xylose, D-adonitol, methyl- β -D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, D-sorbitol, methyl- α -D-mannopyranoside, methyl- α -D-glucopyranoside, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, D-lactose, melibiose, inulin, raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabinol, L-arabinol, potassium

Fig. 1. Phylogenetic tree of representatives of the genera *Brachybacterium*, *Devriesea*, *Dermabacter* and *Helcobacillus* inferred from 16S rRNA gene sequence comparisons (1337 nt fragment) using the neighbour-joining method. Numbers at nodes are proportions of 100 resamplings that support the topology shown. Asterisks after the bootstrap values indicate the nodes that were also obtained in the maximum-parsimony tree. *Corynebacterium amycolatum* was used as the outgroup. Bar, 0.01 nucleotide changes per nucleotide position.

gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. Using API Coryne, the following tests are positive: α -glucosidase, reduction of nitrate, pyrazinamidase, *N*-acetyl- β -glucosaminidase, gelatin hydrolysis, glucose, xylose, mannitol, maltose and sucrose fermentation. The others are negative: β -galactosidase, pyrolidonyl arylamidase, alkaline phosphatase, β -glucuronidase, β -galactosidase, β -glucosidase, urease, ribose, lactose and glycogen fermentation. The compounds used as sole carbon sources recovered when using the Biolog System are: dextrin, *N*-acetyl-D-glucosamine, *N*-acetyl- β -D-mannosamine, L-arabinose, D-fructose, D-galactose, α -D-glucose, m-inositol, maltotriose, D-mannitol, D-mannose (weakly), melezitose, 3-methylglucose, D-ribose, D-sorbitol, sucrose, trehalose, turanose, D-xylose, L-lactic acid, pyruvic acid methyl ester, L-alaninamide, D-alanine, L-alanine, L-alanylglycine, L-asparagine, L-glutamic acid, L-serine, glycerol, adenosine and thymidine. The following are negative: α -cyclodextrin, β -cyclodextrin, glycogen, inulin, mannan, amygdalin, D-arabitol, arbutin, cellobiose, L-fucose, D-galacturonic acid, gentiobiose, D-gluconic acid, α -D-lactose, lactulose, maltose, melibiose, α -methyl-D-galactoside, β -methyl-D-galactoside, α -methyl-D-glucoside, β -methyl-D-glucoside, α -methyl-D-mannoside, palatinose, D-psicose, raffinose, L-rhamnose, salicin, sedoheptulosan, stachyose, D-tagatose, xylitol, acetic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, p-hydroxy-phenylacetic acid, α -ketoglutaric acid, α -ketovaleric acid, lactamide, D-lactic acid methyl ester, D-malic acid, L-malic acid, succinic acid mono-methyl ester, propionic acid, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, 2'-deoxyadenosine, inosine, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, uridine 5'-monophosphate, D-fructose 6-phosphate, α -D-glucose 1-phosphate, D-glucose 6-phosphate and DL- α -glycerol phosphate. Utilization of the following is variable: Tween 40 and Tween 80. The only respiratory quinone is MK-7 and polar lipids present are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and unidentified glycolipids. The major fatty acids are anteiso-C15:0 (34.3%), anteiso-C17:0 (18.7%), iso-C16:0 (18.6%), iso-C14:0 (9%), C18:1 ω 7c and iso-C15:0 (5.2%). Mycolic acids are absent. The isomer of DAP is *meso*-Dpm. Analysis of whole-cell sugars shows high amounts of galactose, ribose and some glucose. The G + C content of the genomic DNA is 68.6 mol%.

The type strain, which was isolated from a human cutaneous discharge sample, is 6401990^T (=CSUR P17^T=CIP 109418^T=CCUG 53859^T).

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