Jonesia quinghaiensis sp. nov., a new member of the suborder *Micrococcineae*

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A coryneform strain isolated from soda lake mud in China corresponded in chemotaxonomic characteristics such as peptidoglycan type A4 α L-Lys–L-Ser–D-Glu and major menaquinone MK-9, as well as in its DNA base composition (57 mol% G + C), to its phylogenetic neighbour *Jonesia denitrificans*. Differences in phenotypic characteristics and the phylogenetic distance (96.6% 16S rRNA gene sequence similarity) from *J. denitrificans* justify the proposal of a second species of the genus *Jonesia*, *Jonesia quinghaiensis* sp. nov., with the type strain QH3A7^T (=DSM 15701^T = CGMCC 1.3459^T).

The only species of the genus *Jonesia*, *Jonesia denitrificans*, type strain CIP 55134^T, was described as a result of a taxonomic study on the genus *Listeria* (Rocourt *et al.* 1987), resulting in the exclusion of *Listeria denitrificans* Prévot 1961 from the genus. Major differences in the DNA G + C value (mol%), peptidoglycan structure, lipid pattern, isoprenoid quinone and the 16S rRNA oligonucleotides supported the taxonomic placement of *J. denitrificans* within the order *Actinomycetales*. The description of the genus *Jonesia* is based on a single isolate. In the present study, a coryneform strain was isolated from soda lake mud in China which showed *Jonesia*-specific characteristics, such as peptidoglycan structure and menaquinone pattern. Based on phenotypic differences and phylogenetic distance from *J. denitrificans*, a second species of the genus *Jonesia*, *Jonesia quinghaiensis* sp. nov., is proposed.

Strain and culture conditions

Strain QH3A7^T was isolated from a mud sample of a soda lake (approx. pH 9) in the west of China. Isolation was done at 28 °C by dilution plating on Bacto marine broth agar (MBA), pH 7.2. The reference strain *J. denitrificans* DSM 20603^T was inoculated on tryptic soy broth agar (TSBA, containing 1.5% Difco agar; Difco) and on MBA, and all morphological and physiological studies were performed with cells grown on these media. For chemotaxonomic

analyses, the organism was grown in tryptic soy broth in flasks on a rotary shaker at 90 r.p.m. and 28 °C. The biomass was harvested by centrifugation, washed twice with distilled water and freeze-dried.

Microscopy and cultural characteristics

The cells were stained after 3–5 days cultivation for Gram's test according to the procedure described by Doetsch (1981). Cells were observed and photographed with a phase-contrast microscope (ZEISS Axiophot) equipped with a Plan-Neofluar objective (100/1.3, oil) and a camera (Sony 3CCD). A micrograph of the Gram-positive cells is shown in Fig. 1.

16S rRNA gene sequence determination and phylogenetic analyses

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were done as described previously by Rainey *et al.* (1996). Electrophoresis of sequencing reaction products was done by using a Beckman CEQ 2000 sequencer according to the manufacturer's protocols. The resulting 16S rRNA gene sequence was compared to sequences from the GenBank database to find the most closely related species. CLUSTAL_X (Thompson *et al.*, 1997) was used to align the QH3A7^T sequence together with those of representatives of each family in the suborder *Micrococcineae* and all members of the family *Jonesiaceae* (Stackebrandt *et al.*, 1997). The resulting 16S rRNA gene alignment was then manually checked by using BioEdit 5.0.9 (Hall, 1999). A dataset of 16 sequences with 1425 nucleotide positions that could be aligned unambiguously was used for calculating the distance

Published online ahead of print on 15 June 2004 as DOI 10.1099/ij.s.0.63223-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain QH3A7^T (=DSM 15701^T = CGMCC 1.3459^T) is AJ626896.

Fig. 1. Micrograph of cells of strain QH3A7^T ($0.5 \times 1.5\text{--}2\text{ }\mu\text{m}$) grown on MBA medium showing rod-shaped cells arranged in threads.

matrix corrected by using Kimura's 2-parameter method (Kimura, 1980). Phylogenetic trees were inferred using the neighbour-joining method (Saitou & Nei, 1987), and bootstrap analysis was based on 1000 resamplings.

Phylogenetic analyses of the almost-complete 16S rRNA gene sequence of strain QH3A7^T (1425 nucleotides) revealed this strain to be closely related to *J. denitrificans* DSM 20603^T (96.6% sequence similarity). The 16S rRNA gene sequence of strain QH3A7^T contained the signature nucleotides of the family *Jonesiaceae* (Stackebrandt & Schumann, 2000). Similarity values with sequences of neighbouring taxa were significantly lower (91.2–93.2%). A dendrogram of relationships (Fig. 2) confirms strains QH3A7^T and DSM 20603^T to be phylogenetic neighbours.

Physiological characteristics

Physiological and biochemical tests were done by using API 50 CHE strips (bioMérieux) according to the manufacturer's instructions. Additionally, Biolog GP2 MicroPlates and the MicroLog computer software (Biolog identification system; Biolog) were used. The growth temperatures (4, 10, 15, 20, 25, 30, 35, 40, 45, 50 °C), pH values (4, 5, 6, 7, 8, 9, 10, 11, 12, 13) and salt tolerance at 2, 5, 7.5, 10, 12.5, 15, 17.5% (w/v) NaCl were determined using MBA. For determination of the pH range, MBA was adjusted to different pH values, after sterilization, using HCl or NaOH.

The results of the physiological tests of strain QH3A7^T and *J. denitrificans* DSM 20603^T are shown in Table 1. The two strains differ from each other in several physiological properties.

Chemotaxonomy

Purified cell-wall preparations were obtained after disruption of cells in a Vibrogen cell mill VI 4 (Johanna Otto GmbH, Germany) by the method of Schleifer (1985). Peptidoglycan structure was elucidated by analyses of cell wall hydrolysates employing the following methods: qualitative analysis of amino acids and peptides by two-dimensional TLC on cellulose plates using solvent systems described previously by Schleifer & Kandler, (1972), quantitative amino acid analysis by GC and GC-MS (MacKenzie, 1987; Groth *et al.*, 1996) and dinitrophenylation of N-terminal amino acids of the interpeptide bridge (Schleifer, 1985). Sugar analysis of the purified cell wall was performed as described by Stanek & Roberts (1974). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Menaquinones

Fig. 2. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic position of strain QH3A7^T, and phylogenetic relatedness between members of the family *Jonesiaceae* and representatives of the families in the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997), order *Actinomycetales*, class *Actinobacteria*. The numbers at the nodes indicate the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets. The scale bar indicates five substitutions per 100 nucleotide positions.

Table 1. Differentiating characteristics of strains QH3A7^T and *J. denitrificans* DSM 20603^T

According to API 50 CHE (*, positive reaction also in the Biolog GP2 substrate panel) both strains utilize: glycerol, L-arabinose*, D-xylose*, galactose, D-glucose*, D-fructose*, D-mannose*, arbutin*, aesculin, salicin*, cellobiose, maltose*, lactose, sucrose*, trehalose, starch, glycogen, β -gentobiose, D-turanose and 5-ketogluconate. Neither strain utilized (†, negative reaction also in the Biolog GP2 substrate panel): erythritol, D-arabinose, ribose, L-xylose, adonitol, methyl β -xyloside, L-sorbose, rhamnose†, dulcitol, inositol†, mannitol†, sorbitol, inulin, melezitose†, D-raffinose†, xylitol, D-tagatose, D-fucose, L-fucose†, D-arabitol†, L-arabitol, 2-ketogluconate, methyl α -D-mannoside†, methyl α -D-glucoside†, N-acetyl-D-glucosamine†. According to the Biolog GP2 substrate panel the following substrates were utilized in addition: dextrin, glycerol, maltotriose, D-psicose, D-ribose, adenosine, 2'-deoxyadenosine. The other substrates of the Biolog GP2 substrate panel were not used. +, Positive; –, negative; W, weakly positive.

Differentiating properties	QH3A7 ^T	<i>J. denitrificans</i> DSM 20603 ^T
API 50 CHE		
Amygdalin	+	–
Melibiose	+	–
D-Lyxose	+	–
Gluconate	+	–
Biolog GP2 MicroPlate		
Mannan	–	W
Amygdalin	+	–
D-Cellobiose	–	+
D-Galactose	–	+
D-Gluconic acid	+	–
Methyl β -D-galactoside	–	W
3-Methyl-glucose	–	+
Palatinose	–	W
D-Sorbitol	–	+
D-Tagatose	–	W
Turanose	–	+
Acetic acid	–	+
Methyl pyruvate	+	–
Propionic acid	–	W
Pyruvic acid	–	W
N-Acetyl-L-glutamic acid	–	W
2,3 Butandiol	–	W
Inosine	–	+
Thymidine	–	+
Uridine	–	+
Motility of cells	Non-motile	Motile‡
Colour of colonies	Yellowish	Greyish, later yellowish‡

‡Data from Seeliger & Jones (1986).

were analysed by HPLC as described previously by Groth *et al.* (1996). Fatty acid methyl esters were extracted and prepared according to the standard protocol of the

Microbial Identification System (MIDI; Microbial ID). Extracts were analysed by using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described previously (Kämpfer & Kroppenstedt, 1996).

The cellular fatty acid pattern consists of 52.8% ai-C15:0, 13.9% i-C16:0, 11.5% C16:0, 5.9% ai-C17:0, 4.8% i-C14:0, 3.8% C14:0, 3.7% i-C15:0, 1.8% ai-C15:1, 0.6% ai-C13:0, 0.6% C15:0, 0.3% C12:0 and 0.3% i-C17:0. Menaquinones were MK-9, MK-8 and MK-7 (peak area ratio, 76:10:10, respectively). Phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and two unknown phospholipids were found as polar lipids. Galactose was detected as the only cell wall sugar. The peptidoglycan of strain QH3A7^T contained the amino acids Lys, Ser, Ala and Glu (1.0:0.9:2.1:2.3 mol, respectively). Glu was found to represent the N terminus of the inter-peptide bridge. From these data and from the occurrence of characteristic di- and tri-peptides in the partial hydrolysate of the peptidoglycan (data not shown), the peptidoglycan type A4 α L-Lys-L-Ser-D-Glu, A11.48 (DSMZ, 2001) was concluded. This peptidoglycan type is identical to that found in *J. denitrificans*. Similarities also exist in the cellular fatty acid, menaquinone and polar lipid patterns (Rocourt *et al.*, 1987; Bernard *et al.*, 1991).

Determination of DNA base composition

DNA was isolated using a French pressure cell and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). The G + C content was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989). The G + C value of 57.3 mol% is within the range of the DNA base content of the genus *Jonesia* (Rocourt *et al.*, 1987).

On the basis of phylogenetic, chemotaxonomic and phenotypic results, we propose strain QH3A7^T to represent a novel species of the genus *Jonesia*, *Jonesia quinghaiensis* sp. nov.

Description of *Jonesia quinghaiensis* sp. nov.

Jonesia quinghaiensis (qing.hai.en'sis, N.L. fem. adj. pertaining to Qinghai, Western province of China, where the type strain was isolated).

Gram-positive, non-acid-fast. On TSBA medium rhizoidal colonies are 4 mm in diameter after 7 days incubation at 28 °C. On MBA medium light-yellowish colonies (about 0.5 mm in diameter) with rhizoid appearance (diameter 4–5 mm). Cells are rod-shaped (0.5 $\times$ 1.5–2 μ m) and non-motile (Fig. 1). Optimal growth temperature is 20–30 °C; optimal pH is 7–9; optimal salt concentration is 2.0–7.5% (w/v) NaCl. The peptidoglycan type is A4 α L-Lys-L-Ser-D-Glu. MK-9 is the predominant menaquinone. The polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and two unknown phospholipids. The major cellular fatty acids are ai-C15:0, i-C16:0

and C16:0. Physiological properties are indicated in Table 1. Isolated from mud of a soda lake in China.

Type strain is QH3A7^T (=DSM 15701^T=CGMCC 1.3459^T).

Acknowledgements

This work was supported by the DSMZ, a scholarship (2002003) from the Yunnan Provincial Department of Education and a grant (30260004) from the National Natural Science Foundation of China (NSFC). We are grateful to Ina Kramer, Anja Frühling and Anika Vester for excellent technical assistance.

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