Jeotgalibacillus soli sp. nov., a Gram-stain-positive bacterium isolated from soil

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A Gram-staining-positive, motile, rod-shaped, spore-forming bacterium, designated P9^T, was isolated from soil in Portugal. This organism was aerobic and catalase- and oxidase-positive. It had an optimum growth temperature of about 35 °C and an optimum growth pH of about 8.0–8.5, and grew in medium with 0–9 % (w/v) NaCl. The cell-wall peptidoglycan was of the A1 α type, with L-lysine as the diagnostic diamino acid. The major respiratory quinone was menaquinone 7 (MK-7) and the major fatty acids were anteiso-C_{15:0} (45.4 %), iso-C_{15:0} (22.0 %) and anteiso-C_{17:0} (11.2 %). The genomic DNA G + C content was about 39.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain P9^T was most closely related to *Jeotgalibacillus campisalis* DSM 18983^T (96.8 %) and *Jeotgalibacillus marinus* DSM 1297^T (96.5 %). These two recognized species formed a coherent cluster with strain P9^T that was supported by a bootstrap value of 99 %. On the basis of the phylogenetic analysis and physiological and biochemical characteristics, strain P9^T (=DSM 23228^T=LMG 25523^T) represents a novel species of the genus *Jeotgalibacillus*, for which the name *Jeotgalibacillus soli* sp. nov. is proposed.

The genus *Jeotgalibacillus* was initially proposed to accommodate organisms that had cell-wall peptidoglycan based on the diamino acid L-lysine, MK-7 and MK-8 as the predominant menaquinones, and anteiso-C_{15:0} as the major fatty acid (Yoon *et al.*, 2001). In a recent taxonomic re-evaluation, it was proposed that species previously assigned to the genus *Marinibacillus*, which also have L-lysine-based peptidoglycan, MK-7 and MK-8 as their predominant menaquinones and anteiso-C_{15:0} as their major fatty acid, should be moved to the genus *Jeotgalibacillus* (Yoon *et al.*, 2010). At the time of writing, the genus *Jeotgalibacillus* comprises four recognized species: *Jeotgalibacillus salarius*, *J. alimentarius*, *J. campisalis* and *J. marinus*. Preliminary phylogenetic analyses based on 16S rRNA gene sequences indicated that a bacterium, designated strain P9^T, that was isolated from soil in Portugal was closely related to these recognized species in the genus *Jeotgalibacillus*. In this study, the morphological, physiological, chemotaxonomic and phylogenetic characteristics of strain P9^T were investigated in detail.

Strain P9^T was isolated on solid ABM2 medium (Tiago *et al.*, 2004) from a sample of alkaline sandy soil (with a pH of

about 9) collected in southern Portugal. This medium contained, per litre: 5 g yeast extract (Difco), 5 g tryptone (Difco), 1 g α -ketoglutaric acid monopotassium salt, 15 g agar (Difco), 50 mmol *N*-cyclohexyl-2-hydroxyl-3-amino-propanesulfonic acid (CAPSO; pH 9.0), 100 ml 10 $\times$ macro-nutrient solution and 10 ml 100 $\times$ micronutrient solution. The 10 $\times$ macronutrient solution contained 1 g nitrilotriacetic acid, 0.6 g CaSO₄·2H₂O, 1 g MgSO₄·7H₂O, 0.8 g NaCl, 1.0 g KNO₃, 6.9 g NaNO₃ and 1.1 g NaHPO₄ 1[–], while the 100 $\times$ micronutrient solution contained 0.2 g MnSO₄·H₂O, 0.05 g ZnSO₄·7H₂O, 0.05 g H₃BO₃, 2.5 mg CuSO₄·5H₂O, 2.5 mg Na₂MoO₄·2H₂O and 4.6 mg CoCl₂·6H₂O 1[–]. The CAPSO buffer and concentrated macro- and micro-nutrient solutions were autoclaved separately from the other components, and the components were only mixed after they had cooled to 50 °C. The soil sample was processed as described by Steele *et al.* (1990). The bacterial isolates were purified by subculture on the same medium and maintained at –70 °C in liquid ABM2 medium (pH 9.0; Tiago *et al.*, 2004) with 15 % (v/v) glycerol. In the characterization of strain P9^T, *J. campisalis* DSM 18983^T and *J. marinus* DSM 1297^T were used for reference.

Unless stated otherwise, all morphological, biochemical and tolerance tests were performed using cultures that were grown, for 24 h at 30 °C, in liquid ABM2 buffered at pH 8.0 with 50 mM TAPS and containing 1 % (w/v) NaCl. Cell morphology, dimensions and motility were examined by

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain P9^T is FR693626.

A supplementary figure and a table are available with the online version of this paper.

phase-contrast light microscopy. Gram staining, flagellum number and location, and cytochrome oxidase and catalase activities were determined as described by Smibert & Krieg (1981). Endospore formation was assessed on two modifications of solid ABM2 medium. Although both modifications to the medium contained 5 mg MnSO₄ l⁻¹, one had the nutrient content reduced to 50% of that in the standard medium and the other had such content reduced to 10% of the standard value (Logan *et al.*, 2009). Cultures of strain P9^T and the type strains of *J. campisalis* and *J. marinus* used for reference were grown on each of these modified media at 30 °C for up to 7 days. The cultures were then checked for endospores as described by Smibert & Krieg (1981). Growth of strain P9^T was followed in liquid ABM2 at 10–50 °C (with intervals of 5 °C) and at 30 °C in the same medium buffered to give pH values between 5.5 and 10.5 (with intervals of 0.5 pH units). The pH range was created using MES, ACES, TAPS, CAPSO or CAPS, as appropriate, each at 50 mM. Salt tolerance was explored in liquid ABM2 with 0–15% (w/v) NaCl (with intervals of 1%). Acid production and enzyme activities were investigated using the API 50CHB/E system

and API ZYM test strips (bioMérieux), respectively, as recommended by the manufacturer. Nitrate reduction, indole production, presence of β-galactosidase and urease, and hydrolysis of gelatin and aesculin were determined using the API 20NE system (bioMérieux), as recommended by the manufacturer. Additionally, the hydrolysis of arginine, elastin, arbutin, gelatin, casein, aesculin, starch and hippurate, and the presence of urease, xylanase and DNase were also assessed, on solid ABM2 for up to 6 days, as described by Hudson *et al.* (1986) and Smibert & Krieg (1981). Results were recorded after 24, 48 and 72 h of incubation at 30 °C.

The cultures for polar lipid analysis were incubated, at 30 °C for 3 days, on solid ABM2 medium buffered at pH 8.0 and containing 1% NaCl. Harvesting of the cultures, extraction of the lipids and two-dimensional TLC, as well as the analysis of respiratory quinones, were performed as described by da Costa *et al.* (2006). Solid ABM2 medium buffered at pH 8.0 and containing 1% NaCl was also used to grow the cells of strain P9^T, *J. campisalis* DSM 18983^T and *J. marinus* DSM 1297^T used

Table 1. Differential phenotypic characteristics of strain P9^T and *J. marinus* DSM 1297^T and *J. campisalis* DSM 18983^T

Taxa: 1, strain P9^T; 2, *J. marinus* DSM 1297^T; 3, *J. campisalis* DSM 18983^T. All results from this study. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Flagellum type	Single polar or subpolar	Peritrichous	Single polar
Catalase	+	–	–
Cytochrome oxidase	+	+	–
Nitrate reduction	–	–	+
Growth with/at:			
0% NaCl	+	–	–
5 °C	–	+	+
40 °C	+	–	–
Hydrolysis of:			
Aesculin	–	+	+
Casein	–	+	+
Gelatin	–	+	–
Urea	+	–	–
Starch	+	–	+
Acid production from:			
Melibiose	–	–	+
Cellobiose	–	–	+
D-Xylose	–	+	–
D-Mannitol	+	–	+
Raffinose	+	–	–
D-Mannose	–	+	–
Enzyme activity (API ZYM)			
Esterase (C4)	w	–	–
Esterase lipase (C8)	w	–	–
Valine arylamidase	w	–	–
β-Galactosidase	–	–	+
α-Glucosidase	+	–	w
Major fatty acids	anteiso-C _{15:0} (45.4%); iso-C _{15:0} (22.0%) and anteiso-C _{17:0} (11.2%)	anteiso-C _{15:0} (40.6%); iso-C _{15:0} (22.1%) and anteiso-C _{17:0} (13.1%)	anteiso-C _{15:0} (46.4%); anteiso-C _{17:0} (11.5%) and C _{16:1} ω7c alcohol (10.2%)

for the fatty acid analyses, the cultures being incubated in a water bath at 30 °C for 3 days. Fatty acid methyl esters (FAME) were obtained from fresh wet biomass by saponification, methylation and extraction, and the fatty acids then identified and quantified with standard MIS Library Generation software (Microbial ID Inc.), as described by the manufacturer. Peptidoglycan analysis was performed, as described previously (Schleifer, 1985), by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig, Germany.

The genomic DNA G+C content of strain P9^T was determined by HPLC (Mesbah *et al.*, 1989) four times, with the mean value and standard deviation calculated. The 16S rRNA gene of the novel strain was sequenced (Rainey *et al.*, 1996) and phylogenetic analyses were performed using the ARB software package (Ludwig *et al.*, 2004). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Felsenstein, 1983) algorithms included in the ARB software package. The topology of the neighbour-joining tree, which was based on evolutionary distances calculated using the Jukes–Cantor correction (Jukes & Cantor, 1969), was evaluated by performing bootstrap analysis (Felsenstein, 1985) with 1000 resamplings of the dataset.

Overall, 27 bacterial strains were isolated from the sample of alkaline sandy soil but only one strain, designated P9^T, appeared to be a novel species in the genus *Jeotgalibacillus*. The other 26 isolates were phylogenetically related to the genera *Arthrobacter*, *Planococcus*, *Kocuria*, *Dietzia* or *Microbacterium*. Strain P9^T formed Gram-staining-positive rod-shaped cells that were motile by means of a polar or subpolar flagellum. This strain produced endospores when grown in medium with low nutrient levels that had been supplemented with 5 mg MnSO₄ l⁻¹. The organism was aerobic and catalase- and oxidase-positive. A more exhaustive description of the physiological characteristics of strain P9^T is given in Table 1, Supplementary Table S1 (available in IJSEM Online) and in the species description. In the analysis of the polar lipids of strain P9^T, four aminophospholipids were visualized on TLC, as also seen with the type strains of *J. campisalis* and *J. marinus* (Supplementary Fig. S1). Strain P9^T possessed a cell-wall peptidoglycan structure of type A1 α (L-lysine). The major respiratory lipoquinone found in strain P9^T was menaquinone 7 (MK-7) and the major fatty acids were anteiso-C_{15:0} (45.4%), iso-C_{15:0} (22.0%) and anteiso-C_{17:0} (11.2%) (Table 2).

The mean ($\pm$ standard deviation) genomic DNA G+C content of strain P9^T was 39.4 $\pm$ 0.8 mol%. A partial sequence from the 16S rRNA gene of strain P9^T, comprising 1525 nt, was determined. Comparison of this sequence with the corresponding sequences of representatives of the main lines of descent within the domain *Bacteria* indicated that strain P9^T was a member of the family *Bacillaceae* (Fig. 1). Strain P9^T formed a coherent cluster, with a bootstrap resampling value of 99%, with the type strains of two species of the genus *Jeotgalibacillus*: *J. campisalis* DSM 18983^T

Table 2. Cellular fatty acid profiles of strain P9^T, *J. marinus* DSM 1297^T and *J. campisalis* DSM 18983^T

Taxa: 1, strain P9^T; 2, *J. marinus* DSM 1297^T; 3, *J. campisalis* DSM 18983^T. All cultures were grown in ABM2 at pH 8 with 1% NaCl and incubated at 30 °C for 3 days before cells were harvested for the preparation of fatty acid methyl esters. All results from this study. Values are percentages of total fatty acids and are presented as the mean $\pm$ standard deviation of four analyses. –, Not detected; tr, trace (<0.5%).

Fatty acid	1	2	3
Straight-chain			
C _{14:0}	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.9 $\pm$ 0.1
C _{15:0}	1.1 $\pm$ 0.1	0.9 $\pm$ 0.1	1.6 $\pm$ 0.1
C _{16:0}	2.3 $\pm$ 0.4	2.1 $\pm$ 0.4	1.3 $\pm$ 0.3
Branched			
iso-C _{14:0}	1.6 $\pm$ 0.4	5.1 $\pm$ 1.6	8.2 $\pm$ 2.9
iso-C _{15:0}	22.0 $\pm$ 0.3	22.1 $\pm$ 1.3	6.8 $\pm$ 1.2
anteiso-C _{15:0}	45.4 $\pm$ 0.9	40.6 $\pm$ 0.1	46.4 $\pm$ 1.5
iso-C _{16:0}	2.5 $\pm$ 0.3	9.4 $\pm$ 0.5	6.1 $\pm$ 0.7
iso-C _{17:0}	5.7 $\pm$ 0.8	4.1 $\pm$ 0.4	0.6 $\pm$ 0.7
anteiso-C _{17:0}	11.2 $\pm$ 0.9	12.95 $\pm$ 0.6	11.5 $\pm$ 2.5
iso-C _{17:1} ω 10c	0.5 $\pm$ 0.3	–	–
Unsaturated			
C _{16:1} ω 7c alcohol	1.8 $\pm$ 0.3	0.8 $\pm$ 0.4	10.2 $\pm$ 2.9
C _{16:1} ω 11c	2.5 $\pm$ 0.2	tr	2.9 $\pm$ 0.1
iso-C _{17:1} I/anteiso B	1.1 $\pm$ 0.8	0.6 $\pm$ 0.4	3.4 $\pm$ 0.7

(96.8% sequence similarity) and *J. marinus* DSM 1297^T (96.5%). These two species were the recognized organisms that appeared to be most closely related to strain P9^T, each showing >96.4% 16S rRNA gene sequence similarity with the novel organism. Strain P9^T shared lower 16S rRNA gene sequence similarity values (about 94.2%) with the type strains of *J. alimentarius* and *J. salarius*.

Several characteristics of strain P9^T, such as its type-A1 α peptidoglycan structure, lipid composition, and fatty acid content (with large amounts of branched fatty acids and a predominance of anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}) were typical of the genus *Jeotgalibacillus*, reinforcing the phylogenetic assignment of the novel strain to this genus. Nevertheless, differences in the proportions of some fatty acids, particularly in the levels of iso-C_{14:0}, iso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0} and C_{16:1} ω 7c alcohol, could be found between the novel isolate and both *J. campisalis* DSM 18983^T and *J. marinus* DSM 1297^T.

Strain P9^T could be differentiated from *J. campisalis* DSM 18983^T and *J. marinus* DSM 1297^T by several phenotypic characteristics, including its growth temperature range, growth in medium with added NaCl, the pattern of acid production from carbohydrates and its enzyme activities. Based on the differential characteristics and on the phylogenetic evidence, strain P9^T is proposed to represent a novel species, for which the name *Jeotgalibacillus soli* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree based on a comparison of the 16S rRNA gene sequences of strain P9^T and its closest, recognized phylogenetic relatives. Bootstrap values (%) for 1000 replicates are given at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated using the maximum-parsimony and maximum-likelihood algorithms. Bar, 0.01 substitution per nucleotide position.

Description of *Jeotgalibacillus soli* sp. nov.

Jeotgalibacillus soli (so'li. L. gen. neut. n. *soli* of or from soil).

Forms rod-shaped (0.8 µm × 1.5–4 µm) cells that are Gram-staining-positive. Cells are convex, without pigmentation, motile by one polar or subpolar flagellum. Endospores, which are central or paracentral, form in diluted growth media supplemented with 5 mg MnSO₄ l⁻¹. The organism is strictly aerobic and oxidase- and catalase-positive. The optimum growth temperature is between 30 and 37 °C, but growth also occurs at 15 and 40 °C. The optimum pH is between 8.0 and 8.5, but growth also occurs at pH 5.5 and pH 10.5. Optimum growth occurs in medium with 0–1 % NaCl (w/v), although growth is still observed in medium with 9 % NaCl. The cell-wall peptidoglycan is of the A1α type, with L-lysine. The main respiratory menaquinone is MK-7. The major fatty acids are anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. Nitrate is not reduced. Starch and urea are hydrolysed, but gelatin, aesculin, casein and Tween 20, Tween 40, Tween 60 and Tween 80 are not hydrolysed. DNase and urease are produced. Xylanase and β-galactosidase are not detected. In API 50CHB/E tests, acid is produced from glycerol, D-ribose, D-glucose, D-fructose, D-mannitol, N-acetylglucosamine, maltose, sucrose, trehalose, raffinose, starch, glycogen and inulin, but not from melibiose, melezitose, cellobiose, D-xylose, L-xylose, D-mannose, L-rhamnose, D-arabitol, L-arabitol, erythritol, D-galactose, D-adonitol, methyl β-D-xylopyranoside, D-arabinose, L-arabinose, L-sorbose, sorbitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, lactose, xylitol, gentiobiose, turanose, L-lyxose, D-tagatose, D-fucose, L-fucose, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate.

The type strain, P9^T (=DSM 23228^T=LMG 25523^T), was isolated from soil in Portugal. The genomic DNA G+C content of the type strain is about 39.4 mol% (HPLC method).

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