Lysinibacillus cresolivorans sp. nov., an *m*-cresol-degrading bacterium isolated from coking wastewater treatment aerobic sludge

Yuan Ren,[1,2,3] Shao-yi Chen,[1] Hai-yan Yao[1] and Liu-jie Deng[1]

Correspondence
Yuan Ren
ceyren@scut.edu.cn

[1]School of Environment and Energy, South China University of Technology, Panyu District, Guangzhou 510006, PR China

[2]Key Lab of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education, PR China

[3]Key Laboratory of Environmental Protection and Eco-Remediation of Guangdong Regular Higher Education Institutions, PR China

A Gram-stain-positive, rod-shaped, facultatively anaerobic, endospore-forming bacterium (designated strain SC03^T) was isolated from the aerobic treatment sludge of a coking plant (Shaoguan City, China). The optimal pH and temperature for growth were pH 7.0 and 35 °C. On the basis of 16S rRNA gene sequence analysis, strain SC03^T was related to the genus *Lysinibacillus* and the similarity between strain SC03^T and the most closely related type strain, *Lysinibacillus macroides* LMG 18474^T, was 94.4 %. The genomic G+C content of the DNA of strain SC03^T was 41.2 mol%. Chemotaxonomic data supported the affiliation of strain SC03^T to the genus *Lysinibacillus*. These properties include MK-7 as the predominant menaquinone; iso-$C_{15:0}$ and iso-$C_{16:0}$ as major fatty acids; A4α (L-Lys–D-Asp) as the cell-wall peptidoglycan type; and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine plus three unknown phospholipids as polar lipids. The phenotypic, phylogenetic and chemotaxonomic characters enable the differentiation of strain SC03^T from recognized *Lysinibacillus* species. Thus, strain SC03^T represents a novel species of the genus *Lysinibacillus*, for which the name *Lysinibacillus cresolivorans* sp. nov. is proposed. The type strain is SC03^T (=NRRL B-59352^T=CCTCC M 208210^T).

Species of the genus *Bacillus* are endospore-forming bacteria, most of which are rod-shaped, oval or round with strong resistance to adverse environmental conditions. They are very common in soil, water, air and the intestinal tract of animals. Ash *et al.* (1991) first separated members of the genus *Bacillus* into five clusters based on rRNA gene sequence analysis, and the genus has since been split into several genera. The genus *Lysinibacillus* was first proposed by Ahmed *et al.* (2007) with the description of a novel species and re-examination of *Bacillus* rRNA group 2 containing lysine and aspartic acid in the peptidoglycan of the cell wall. At the time of writing, the genus *Lysinibacillus* consists of 18 recognized species: *Lysinibacillus boronitolerans*, *L. fusiformis*, *L. sphaericus* (Ahmed *et al.*, 2007), *L. parviboronicapiens* (Miwa *et al.*, 2009), *L. xylanilyticus* (Lee *et al.*, 2010), *L. macroides* (Coorevits *et al.*, 2012), *L. sinduriensis*, *L. massiliensis*, *L. odysseyi* (Jung *et al.*, 2012), *L. mangiferahumi* (Yang *et al.*, 2012), *L. meyeri* (Seiler *et al.*, 2013), *L. manganicus* (Liu *et al.*, 2013), *L. contaminans* (Kämpfer *et al.*, 2013), *L. varians* (Zhu *et al.*, 2014), *L. tabacifolii* (Duan *et al.*, 2013), *L. halotolerans* (Kong *et al.*, 2014), *L. fluoroglycofenilyticus* (Cheng *et al.*, 2015) and *Lysinibacillus acetophenoni* (Azmatunnisa *et al.*, 2015). These species are characterized as endospore-forming, motile rods, with an A4α-type (Lys–Asp) peptidoglycan cell wall, iso-$C_{15:0}$ or anteiso-$C_{15:0}$ as the predominant cellular fatty acid, MK-7 as the major menaquinone, and a genomic G+C content of 35–43.2 mol% (Liu *et al.*, 2013; Zhu *et al.*, 2014). During a study of *m*-cresol-degrading bacteria (Zhao *et al.*, 2014), strain SC03^T was isolated from aerobic sludge of a coking wastewater treatment plant located in Guangdong Province, China. In this paper, we report the taxonomic properties of strain SC03^T, an endospore-forming Gram-stain-positive bacterium, as a novel member of the genus *Lysinibacillus*. To describe this bacterium, the minimal standards for describing new taxa of aerobic, endospore-forming

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SC03^T is EU043375.

One supplementary table and two supplementary figures are available with the online Supplementary Material.

bacteria (Logan *et al.*, 2009) were followed to determine the morphological, physiological, biochemical and chemotaxonomic characteristics.

To screen for *m*-cresol-degrading bacteria, coking wastewater treatment sludge, which contains more than 15 different phenolic compounds, was collected, aerated for 48 h and the supernatant then used as inoculum. The isolation medium contained (per litre): 2.24 g K_2HPO_4, 2.75 g KH_2PO_4, 1.00 g $(NH_4)_2SO_4$, 0.2 g $MgCl_2 \cdot 6H_2O$, 0.1 g NaCl, 0.02 g $FeCl_3 \cdot 6H_2O$ and 0.01 g $CaCl_2$ (pH 7.0–7.2) (Yao *et al.*, 2011). *m*-Cresol was added at 50 mg ml^{-1} via a 0.22 μm membrane filter to a 100 ml mixture of sludge supernatant and sterile medium (1 : 9, v/v). After initial growth was observed by an increase in turbidity, 10 ml of the suspension was transferred into a fresh sterile isolation medium containing 100 mg l^{-1} *m*-cresol. This procedure was repeated six times until the concentration of *m*-cresol was increased to 500 mg l^{-1}. The final enriched culture was spread on agar plates with 200 mg l^{-1} *m*-cresol as the sole carbon source. After incubation for 24 h, different colonies were selected and isolated using the streak plating method three times on agar plates at 35 °C. One isolate, designated strain SC03^T, was purified for further characterization. The origin of the strain is from the aerobic sludge of a coking plant (Shaoguan City, China; 24° 42′ 21.99″ N 113° 38′ 46.46″ E). *L. macroides* DSM 54^T, *L. boronitolerans* DSM 17140^T, *L. xylanilyticus* KCTC 13423^T, *L. fusiformis* KACC 10903^T and *L. sphaericus* KCTC 3346^T were used as reference strains in this study and all the testing was conducted using fresh cultures of purified strains under the same conditions.

The biodegradation abilities of the new isolate and the reference strains were tested with *m*-cresol as sole carbon and energy source in the isolation medium. First, all strains were activated under optimal conditions and one loop of each culture was inoculated into the enrichment culture medium in 100 ml flasks at 35 °C. Next, the bacterial suspensions were inoculated into the medium with 20, 50 or 100 mg l^{-1} *m*-cresol as sole carbon and energy source. All cultures were placed into flasks, which were incubated on a horizontal shaker at 28 °C for 72 h. The concentrations of *m*-cresol were measured with an HPLC system after centrifugation of the liquid culture to estimate the biodegradation by the strains. Strain SC03^T and the reference strains were able to degrade *m*-cresol at 20 mg l^{-1}. As shown in Table 1, when the initial concentration of *m*-cresol was increased to 50 mg l^{-1}, it was degraded by strain SC03^T, *L. boronitolerans* DSM 17140^T and *L. xylanilyticus* KCTC 13423^T. Among these strains, only

Table 1. Phenotypic characteristics of strain SC03^T and related *Lysinibacillus* species

Strains: 1, SC03^T; 2, *L. macroides* DSM 54^T; 3, *L. boronitolerans* DSM 17140^T; 4, *L. xylanilyticus* KCTC 13423^T; 5, *L. fusiformis* KACC 10903^T; 6, *L. sphaericus* KCTC 3346^T. Data are from this study, except for the utilization of different carbon sources for *L. fusiformis* and *L. sphaericus*, which are taken from Priest *et al.* (1988). +, Positive; −, negative.

Characteristic	1	2	3	4	5	6
Cell length (μm)	3.3–5.0	1.4–2.0	2.8–3.0	1.8–4.5	2.0–4.0	1.4–4.8
Cell diameter (μm)	1.1–1.3	0.8–0.9	1.1–1.2	0.9–1.2	0.5–1.5	0.6–1.0
Endospore shape and position*	O,T	R,T	R/O,T	R,T	R/C,T	R,T
Temperature range for growth (°C)	25–40	20–45	15–45	10–40	15–40	10–40
pH range for growth	5.0–9.5	6.5–9.5	5.0–9.5	5.0–9.5	6.0–9.5	6.0–9.0
Anaerobic growth	+	−	−	−	−	−
NaCl range for growth (%)	0–5	0.5–4	0–4	0–4	0–7	0–4
Voges–Proskauer test	−	+	−	−	−	−
Nitrate reduction	+	+	+	+	−	−
Hydrolysis of:						
Gelatin	−	−	−	+	−	−
Urea	+	−	+	−	−	−
Aesculin	−	+	+	+	+	+
Utilization of:						
Citrate	−	+	+	+	+	+
Tween 40	+	+	−	−	+	+
Acetate	+	−	−	−	+	+
α-Ketoglutaric acid	−	−	−	−	+	+
L-Malic acid	−	+	+	+	−	−
Biodegradation of *m*-cresol at:						
50 mg l^{-1}	+	−	+	+	−	−
100 mg l^{-1}	+	−	+	−	−	−

*C, Central; O, oval or slightly oval; R, round; T, terminal.

SC03^T and *L. boronitolerans* DSM 17140^T were able to degrade *m*-cresol at 100 mg l^{-1}.

The cell morphological and motility features were observed using light microscopy (Eclipse E200; Nikon) with cells grown for 48 h at 28 °C on tryptone soy agar (TSA; Bioway). Cell form and flagellation were observed using the phosphotungstic acid staining method (for 1.5 min) with a transmission electron microscope (H-7650; Hitachi). Growth of the strain at different temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C), NaCl concentrations (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 %) and pH (range 4–10, with an interval of 0.5 pH units; CH_3COONa-CH_3COOH buffer for pH 4–5, K_2HPO_4-KH_2PO_4 buffer for pH 5.5–8 and $NaHCO_3$-NaOH buffer for pH 8.5–10) was determined on nutrient agar (NA) plates for 1 week. The Gram reaction was performed as described by Gregersen (1978). Enzyme activity, hydrolysis reactions, catalase activity, oxidase activity, indole production, and nitrate and nitrite reduction were determined by standard methods (Chang *et al.*, 2002; Smibert & Krieg, 1994). Growth under anaerobic conditions was determined after 2 weeks of incubation. The Voges–Proskauer test was determined in Voges–Proskauer broth for 6–10 days. Resistance to antibiotics was assessed using the Oxford cup method (Abraham *et al.*, 1941) at the following concentrations: penicillin, 10 μg; chloromycetin, 30 μg; streptomycin, 10 μg; tetracycline, 30 μg; and rifampicin, 5 μg. Liquefaction of gelatin was tested on peptone gelatin medium at 20 °C using the new isolate and related strains grown for 18–24 h. Utilization of carbon sources and other organic compounds was tested using the GP2 MicroPlate system (Biolog) according to the manufacturer's instructions.

A transmission micrograph of cells of strain SC03^T is shown in Fig. S1 (available in the online Supplementary Material). Cells of the strain produced oval endospores in the terminal position in a swollen sporangium. Strain SC03^T grew at pH 5.0–9.5 with optimum growth at pH 7.0 and no growth at pH 4.5. This pH range is similar to those of most species of *Lysinibacillus*, with the exception of *L. contaminans*, which can even tolerate pH 11.5 (Kämpfer *et al.*, 2013). Growth of strain SC03^T occurred at 25–40 °C with optimum growth at 35 °C; there was no growth at ≥45 °C and weak growth after several days at 20 °C. Strain SC03^T tolerated 0–5 % (w/v) NaCl on NA plates. Nearly all members of the genus *Lysinibacillus* have this NaCl tolerance range, except for *L. fusiformis*, which can tolerate up to 7 % (Priest *et al.*, 1988). Strain SC03^T was resistant to tetracycline, chloromycetin and rifampicin, but sensitive to penicillin and streptomycin. Other differential phenotypic characteristics are listed in Table 1.

Genomic DNA of strain SC03^T was extracted using the method described by Zhou *et al.* (1996) and the nearly complete 16S rRNA gene sequence was amplified using primers F27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and R1522 (5′-AAGGAGGTGATCCAGCCGCA-3′). The purified PCR product (1452 nt) was sequenced by Shanghai Bioasia (Invitrogen) Biotechnology. The EzTaxon-e server was used to analyse the sequences (Kim *et al.*, 2012). Phylogenetic trees of strain SC03^T and related taxa were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and minimum-evolution (Rzhetsky & Nei, 1992) algorithms with 1000 bootstrap replications (Felsenstein, 1985) using MEGA version 4.0 software (Kumar *et al.*, 2004) after alignment of the data using CLUSTAL X (Thompson *et al.*, 1997).

The nearly complete 16S rRNA gene sequence for strain SC03^T was used to reconstruct phylogenetic trees. Comparative 16S rRNA gene sequence analysis placed strain SC03^T within the genus *Lysinibacillus*. Strain SC03^T showed highest similarity to the sequence of *L. macroides* LMG 18474^T (94.4 %), *L. boronitolerans* 10a^T (94.0 %), *L. xylanilyticus* XDB9^T (93.5 %), *L. fusiformis* NBRC 15717^T (93.4 %) and *L. sphaericus* KCTC 3346^T (93.2 %), and lower sequence similarity (<93 %) to other type strains of *Lysinibacillus* species. Based on the 16S rRNA gene sequence phylogeny, strain SC03^T branched closest to *L. boronitolerans* 10a^T and *L. macroides* LMG 18474^T. The relationship among strain SC03^T, *L. boronitolerans* 10a^T and *L. macroides* LMG 18474^T was similar in the trees built using the maximum-parsimony and minimum-evolution algorithms and was supported by high bootstrap values (Fig. 1).

L. boronitolerans DSM 17140^T, *L. xylanilyticus* KCTC 13423^T and *L. macroides* DSM 54^T were used to assay the fatty acids in comparison with strain SC03^T. For cellular fatty acid analysis, cell biomass was harvested from TSA plates after cultivation for 24 h at 28 °C. Three to four inoculation loops of cell material were collected from the plates and used for the analysis. The cellular fatty acid composition of the strain was examined by GC using the Microbial Identification system (MIDI; version 4.0) according to the manufacturer's instructions. The genomic G+C content of the DNA was determined using HPLC according to Mesbah *et al.* (1989).

Cell biomass for the analysis of cell-wall components, polar lipids and respiratory quinones was harvested from cultures grown on TSA at 30 °C for 48 h. The menaquinones were extracted and investigated according to the method described by Collins (1981) and Groth *et al.* (1996) via HPLC. A purified cell-wall preparation was obtained and hydrolysed as described by Schleifer & Kandler (1972). The cell-wall peptidoglycan was obtained and purified according to the description given by Schleifer (1985). The amino acid contents of the cell wall were determined by HPLC according to Tang *et al.* (2009). Polar lipids were extracted and identified using two-dimensional TLC (Minnikin *et al.*, 1979; Collins & Jones, 1980) after staining with molybdatophosphoric acid.

The major menaquinone of strain SC03^T was MK-7 (89.2 %), and a minor amount of MK-6 (10.8 %) was

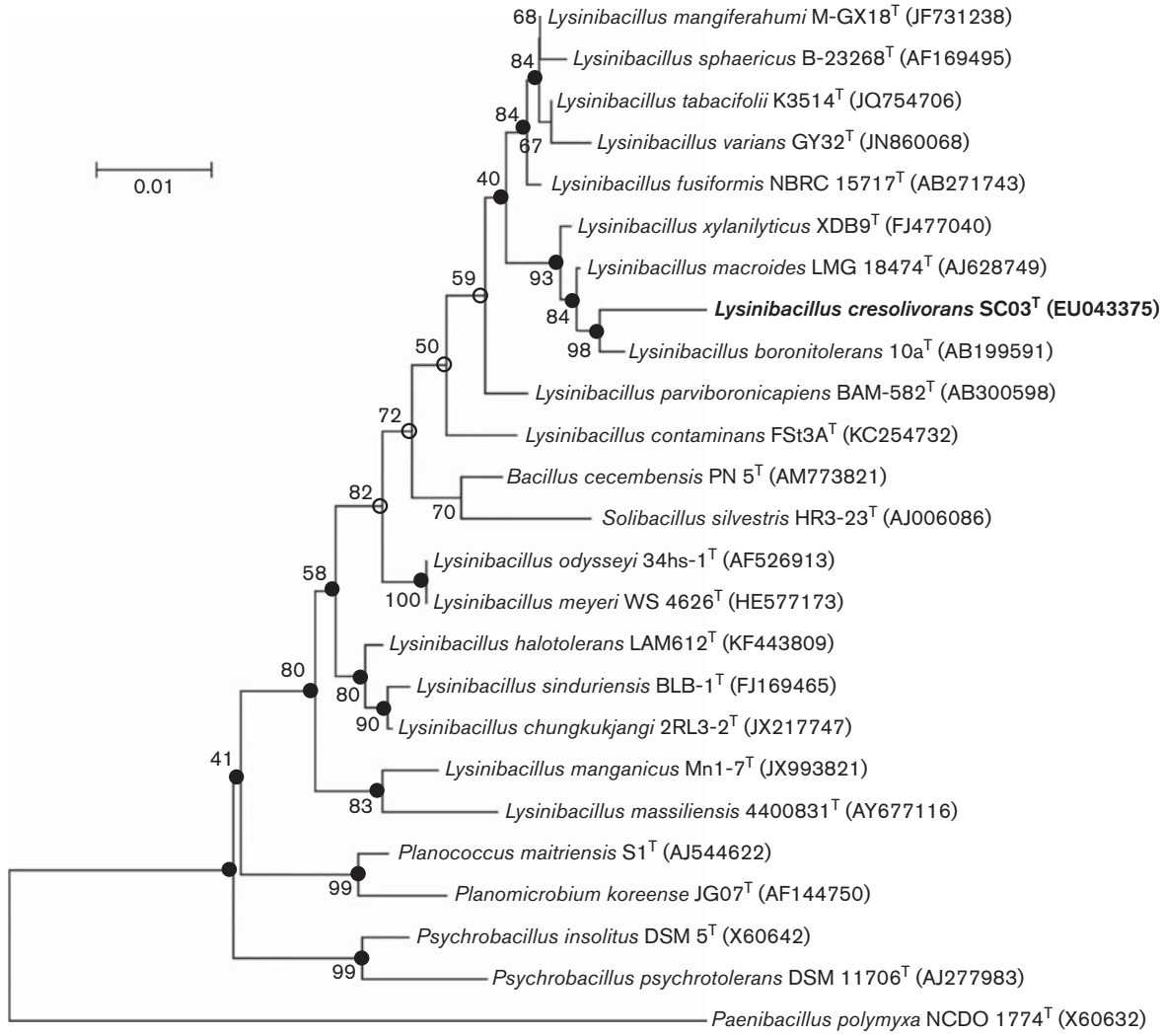

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences, demonstrating the phylogenetic position of strain SC03^T and closely related *Lysinibacillus* species. *Paenibacillus polymyxa* NCDO 1774^T (X60632) was used as the outgroup. Bootstrap values at the nodes are based on 1000 replications. Filled circles indicate nodes that were obtained by all treeing methods (neighbour-joining, minimum-evolution and maximum-parsimony) and open circles nodes that were recovered by the neighbour-joining and minimum-evolution methods. Bar, 1 substitution per 100 nt.

detected as well; this corresponds to what has been reported previously for the genus (Ahmed *et al.*, 2007). The major cellular fatty acids of strain SC03^T were iso-$C_{15:0}$ (59.5 %), iso-$C_{16:0}$ (13.5 %) and anteiso-$C_{15:0}$ (8.6 %). Strain SC03^T shared the same major fatty acids as recognized species of the genus *Lysinibacillus*; however, the relative abundances of these cellular fatty acids were distinctly different (Table S1). Thus, the fatty acid profiles can be used to differentiate these species. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, while three unknown phospholipids were also detected in minor amounts (Fig. S2). This result is consistent with other *Lysinibacillus* species except for *L. massiliensis* (Jung *et al.*, 2012). Compared with the polar lipid profiles of other *Lysinibacillus* species, strain SC03^T showed a very similar pattern to those of *L. odysseyi*, *L. sinduriensis* (Jung *et al.*, 2012) and *L. contaminans* (Kämpfer *et al.*, 2013). The genomic G+C content of the DNA of strain SC03^T was 41.2 mol%; other *Lysinibacillus* species have genomic G+C contents of 35–43.2 mol% (Liu *et al.*, 2013; Zhu *et al.*, 2014).

From quantitative analysis of the cell-wall peptidoglycan, strain SC03^T contained alanine, glutamic acid, aspartic acid and lysine in a molar ratio of 1.03 : 1.0 : 0.79 : 0.97. Peptidoglycan type A4α (Lys–Asp) is a key marker for distinguishing the genus *Lysinibacillus* from other members of *Bacillus* group 2, with the above diagnostic amino acids at ratios of 1.83 : 1.0 : 0.63 : 0.69 for *L. boronitolerans* DSM

17140^T (Ahmed *et al.*, 2007). Data have also been published for *L. parviboronicapiens* KCTC 13154^T (1.93 : 1.0 : 0.89 : 0.86) (Miwa *et al.*, 2009), *L. xylanilyticus* KCTC 13423^T (1.43 : 1.0 : 0.43 : 0.33) (Lee *et al.*, 2010), *L. sinduriensis* KCTC 13296^T (1.3 : 1.0 : 1.0 : 0.9) (Jung *et al.*, 2012), *L. meyeri* DSM 25027^T (1.5 : 1.0 : 0.8 : 0.9) (Seiler *et al.*, 2013) and *L. contaminans* DSM 25560^T (1.6 : 1.0 : 0.9 : 0.8) (Kämpfer *et al.*, 2013). Strain SC03^T exhibited a ratio that most closely matched *L. meyeri*, and all these species of *Lysinibacillus* share similar peptidoglycan composition ratios according to Schleifer & Kandler (1972).

The phenotypic and phylogenetic results obtained clearly indicate that strain SC03^T represents a novel species within the genus *Lysinibacillus*, for which the name *Lysinibacillus cresolivorans* sp. nov. is proposed.

Description of *Lysinibacillus cresolivorans* sp. nov.

Lysinibacillus cresolivorans (cre.so.li.vo′rans. N.L. n. *cresololis* cresol; L. part. adj. *vorans* devouring; N.L. part. adj. *cresolivorans* cresol-degrading).

Cells are Gram-stain-positive, motile rods, 1.1–1.3 × 3.3–5.0 μm in size. Endospores are oval in the terminal position within a swollen sporangium. Colonies are cream-pigmented, with irregular margins, rough, opaque and with a matte surface on NA, and are 0.8–2.0 mm in diameter after 48 h at 30 °C. In liquid culture medium, cells aggregate and settle to the bottom of the tube. Facultatively anaerobic; very weak growth is observed when cultivated under anaerobic conditions. The temperature range for growth is 25–40 °C, with optimum growth at 35 °C; there is no growth at ≥45 °C. Growth is observed at pH 5.0–9.5, with optimum growth at pH 7.0. The NaCl tolerance range is 0–5 % (w/v) on NA. Catalase-positive and oxidase-negative. Negative results are obtained for the Voges–Proskauer test, the methyl red test, hydrolysis of starch, gelatin, casein and aesculin, phenylalanine deaminase, arginine dihydrolase, lysine decarboxylase and lecithinase, the production of indole and H_2S, and nitrite reduction. Positive for nitrate reduction, hydrolysis of urea and Tween 80. Acid is not produced from D-glucose, L-arabitol, D-mannitol or D-xylose. The following compounds can be utilized: cyclodextrin, Tween 40, D-ribose, acetic acid, α-ketovaleric acid, succinic acid monomethyl ester, L-alaninamide, L-alanine, L-serine, 2,3-butanediol, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, thymidine-5′-monophosphate and uridine-5′-monophosphate. MK-7 is the predominant menaquinone. The major cellular fatty acids (>10 %) are iso-$C_{15:0}$ and iso-$C_{16:0}$. The cell-wall peptidoglycan type is A4α (L-Lys–D-Asp). The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine.

The type strain, SCO3^T (=NRRL B-59352^T=CCTCC M 208210^T), was isolated from a coking wastewater treatment plant in Shaoguan, China. The genomic G+C content of the DNA of the type strains is 41.2 mol%.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant nos. 20977035, 51178190), Research Fund of SIT of Guangzhou, and Research Fund of The Key Lab of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education, China. We thank Dr Max Häggblom for help with the linguistic revision and technical suggestions. We also thank Prof. Jean P. Euzéby for the nomenclature of this strain.

References

Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner, A. D., Heatley, N. G. & Jennings, M. A. (1941). Further observations on penicillin. *Lancet* **2**, 177–189.

Ahmed, I., Yokota, A., Yamazoe, A. & Fujiwara, T. (2007). Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. *Int J Syst Evol Microbiol* **57**, 1117–1125.

Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.

Azmatunnisa, M., Rahul, K., Lakshmi, K. V. N. S., Sasikala, Ch. & Ramana, Ch. V. (2015). *Lysinibacillus acetophenoni* sp. nov., a solvent-tolerant bacterium isolated from acetophenone. *Int J Syst Evol Microbiol* **65**, 1741–1748.

Chang, Y. H., Han, J. I., Chun, J., Lee, K. C., Rhee, M. S., Kim, Y. B. & Bae, K. S. (2002). *Comamonas koreensis* sp. nov., a non-motile species from wetland in Woopo, Korea. *Int J Syst Evol Microbiol* **52**, 377–381.

Cheng, M., Zhang, H., Zhang, J., Hu, G., Zhang, J., He, J. & Huang, X. (2015). *Lysinibacillus fluoroglycofenilyticus* sp. nov., a bacterium isolated from fluoroglycofen contaminated soil. *Antonie van Leeuwenhoek* **107**, 157–164.

Collins, M. D. (1981). Distribution of menaquinones within members of the genus *Staphylococcus*. *FEMS Microbiol Lett* **12**, 83–85.

Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. *J Appl Microbiol* **48**, 459–470.

Coorevits, A., Dinsdale, A. E., Heyrman, J., Schumann, P., Van Landschoot, A., Logan, N. A. & De Vos, P. (2012). *Lysinibacillus macroides* sp. nov., nom. rev. *Int J Syst Evol Microbiol* **62**, 1121–1127.

Duan, Y. Q., He, S. T., Li, Q. Q., Wang, M. F., Wang, W. Y., Zhe, W., Cao, Y. H., Mo, M. H., Zhai, Y. L. & Li, W. J. (2013). *Lysinibacillus tabacifolii* sp. nov., a novel endophytic bacterium isolated from *Nicotiana tabacum* leaves. *J Microbiol* **51**, 289–294.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gregersen, T. (1978). Rapid method for distinction of gram-negative from gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Jung, M. Y., Kim, J., Paek, W. K., Styrak, I., Park, I., Sin, Y., Paek, J., Park, K. A., Kim, H. & other authors (2012). Description of *Lysinibacillus sinduriensis* sp. nov., and transfer of *Bacillus massiliensis*

and *Bacillus odysseyi* to *Lysinibacillus* as *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odysseyi* comb. nov. with emended description of the genus. *Int J Syst Evol Microbiol* **62**, 2347–2356.

Kämpfer, P., Martin, K. & Glaeser, S. P. (2013). *Lysinibacillus contaminans* sp. nov., isolated from surface water. *Int J Syst Evol Microbiol* **63**, 3148–3153.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kong, D., Wang, Y., Zhao, B., Li, Y., Song, J., Zhai, Y., Zhang, C., Wang, H., Chen, X. & other authors (2014). *Lysinibacillus halotolerans* sp. nov., isolated from saline-alkaline soil. *Int J Syst Evol Microbiol* **64**, 2593–2598.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Lee, C. S., Jung, Y. T., Park, S., Oh, T. K. & Yoon, J. H. (2010). *Lysinibacillus xylanilyticus* sp. nov., a xylan-degrading bacterium isolated from forest humus. *Int J Syst Evol Microbiol* **60**, 281–286.

Liu, H., Song, Y., Chen, F., Zheng, S. & Wang, G. (2013). *Lysinibacillus manganicus* sp. nov., isolated from manganese mining soil. *Int J Syst Evol Microbiol* **63**, 3568–3573.

Logan, N. A., Berge, O., Bishop, A. H., Busse, H. J., De Vos, P., Fritze, D., Heyndrickx, M., Kämpfer, P., Rabinovitch, L. & other authors (2009). Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* **59**, 2114–2121.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Evol Microbiol* **39**, 159–167.

Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas, Oerskovia* and related taxa. *J Appl Microbiol* **47**, 87–95.

Miwa, H., Ahmed, I., Yokota, A. & Fujiwara, T. (2009). *Lysinibacillus parviboronicapiens* sp. nov., a low-boron-containing bacterium isolated from soil. *Int J Syst Evol Microbiol* **59**, 1427–1432.

Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* **134**, 1847–1882.

Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* **9**, 945–967.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

Seiler, H., Scherer, S. & Wenning, M. (2013). *Lysinibacillus meyeri* sp. nov., isolated from a medical practice. *Int J Syst Evol Microbiol* **63**, 1512–1518.

Smibert, R. & Krieg, N. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. Murray, W. Wood & N. Krieg. Washington, DC: American Society for Microbiology.

Tang, S. K., Wang, Y., Chen, Y., Lou, K., Cao, L. L., Xu, L. H. & Li, W. J. (2009). *Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. *Int J Syst Evol Microbiol* **59**, 2025–2031.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Yang, L. L., Huang, Y., Liu, J., Ma, L., Mo, M. H., Li, W. J. & Yang, F. X. (2012). *Lysinibacillus mangiferahumi* sp. nov., a new bacterium producing nematicidal volatiles. *Antonie van Leeuwenhoek* **102**, 53–59.

Yao, H., Ren, Y., Wei, C. & Yue, S. (2011). Biodegradation characterisation and kinetics of *m*-cresol by *Lysinibacillus cresolivorans*. *Water SA* **37**, 15–20.

Zhao, G., Chen, S. Y., Ren, Y. & Wei, C. (2014). Interaction and biodegradation evaluate of *m*-cresol and quinoline co-exist system. *Int Biodeter Biodegr* **86**, 252–257.

Zhou, J., Bruns, M. A. & Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. *Appl Environ Microb* **62**, 316–322.

Zhu, C., Sun, G., Chen, X., Guo, J. & Xu, M. (2014). *Lysinibacillus varians* sp. nov., an endospore-forming bacterium with a filament-to-rod cell cycle. *Int J Syst Evol Microbiol* **64**, 3644–3649.