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Lysobacter ruishenii sp. nov., a chlorothalonil-degrading bacterium isolated from a long-term chlorothalonil-contaminated soil

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An aerobic, Gram-negative bacterial strain, designated CTN-1^T, capable of degrading chlorothalonil was isolated from a long-term chlorothalonil-contaminated soil in China, and was subjected to a polyphasic taxonomic investigation. Strain CTN-1^T grew at 15–37 °C (optimum 28–30 °C) and at pH 6.0–9.0 (optimum pH 7.0–7.5). The G+C content of the total DNA was 67.1 mol%. Based on 16S rRNA gene sequence analysis, strain CTN-1^T was related most closely to *Lysobacter daejeonensis* DSM 17634^T (97.1 % similarity), *L. soli* DCY21^T (95.7 %), *L. concretionis* Ko07^T (95.5 %), *L. gummosus* LMG 8763^T (95.3 %) and *L. niastensis* DSM 18481^T (95.2 %). The novel strain showed less than 95.0 % 16S rRNA gene sequence similarity to the type strains of other *Lysobacter* species. The major cellular fatty acids of strain CNT-1^T were iso-$C_{16:0}$ (23.0 %), iso-$C_{15:0}$ (21.4 %) and iso-$C_{17:1}\omega9c$ (15.3 %). The major isoprenoid quinone was Q-8 (99 %), and the major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. These chemotaxonomic data supported the affiliation of strain CTN-1^T to the genus *Lysobacter*. Levels of DNA–DNA relatedness between strain CTN-1^T and *L. daejeonensis* DSM 17634^T were 34.6–36.1 %. Phylogenetic analysis based on 16S rRNA gene sequences, DNA–DNA hybridization data and biochemical and physiological characteristics strongly supported the genotypic and phenotypic differentiation of strain CTN-1^T from recognized species of the genus *Lysobacter*. Strain CTN-1^T is therefore considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter ruishenii* sp. nov. is proposed. The type strain is CTN-1^T (=DSM 22393^T =CGMCC 1.10136^T).

The genus *Lysobacter*, grouped in the family *Xanthomonadaceae*, is classified in the class *Gammaproteobacteria* (Christensen & Cook, 1978) based on non-fruiting bodies, lack of flagella, gliding nature and high genomic DNA G+C content (typically ranging between 65.4 and 70.1 mol%) (Aslam *et al.*, 2009). Species of the genus *Lysobacter* have been described as ubiquitous inhabitants of soil and water, and are commonly found in diverse geographical and environmental habitats (Romanenko *et al.*, 2008; Yassin *et al.*, 2007). At the time of writing, the genus *Lysobacter* comprises 16 recognized species, four described since 2009 (Aslam *et al.*, 2009; Srinivasan *et al.*, 2010; Ten *et al.*, 2009; Wang *et al.*, 2009). Members of the genus were found to have great potential for the development of biocontrol agents against plant fungal pathogens (Islam *et al.*, 2005; Park *et al.*, 2008) and antibiotic compounds against human pathogens (Ahmed *et al.*, 2003; Hashizume *et al.*, 2004). However, to the best of our knowledge, members of the genus *Lysobacter* with the ability to degrade xenobiotics have not been reported.

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), a broad-spectrum chlorinated aromatic fungicide, is the second most widely used agricultural fungicide in the United States, with 5×10^6 kg applied annually (Cox, 1997). Chlorothalonil is highly toxic to fish, birds and aquatic invertebrates (Caux *et al.*, 1996) and is commonly detected in ecosystems (Kazos *et al.*, 2008). During the isolation of chlorothalonil-degrading micro-organisms, a bacterial strain (designated CTN-1^T) capable of efficiently degrading chlorothalonil without the presence of other carbon sources was characterized from a long-term chlorothalonil-contaminated soil at the Jiangyin Suli Chemical Co., Ltd

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CTN-1^T is GU086401.

Three supplementary figures and a supplementary table are available with the online version of this paper.

(Jiangsu, China). Strain CTN-1^T was characterized by a polyphasic approach, including determination of chemotaxonomic and phenotypic properties, phylogenetic analysis based on 16S rRNA gene sequences and genomic relatedness. On the basis of the results obtained, we propose that strain CTN-1^T should be placed in the genus *Lysobacter* as the type strain of a novel species.

The isolation of strain CTN-1^T was carried out by enrichment culture with minimal salt medium (per litre distilled water: 1.0 g NH_4NO_3, 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.2 g $MgSO_4$ and 1.0 g NaCl, pH 7.0) supplemented with 50 mg chlorothalonil l^{-1}. Chlorothalonil (99.3 % purity) was purchased from Sigma-Aldrich. The enrichment culture was spread on Luria–Bertani (LB) agar (Difco) containing 100 mg chlorothalonil l^{-1}. Colonies with clear transparent zones due to chlorothalonil degradation were selected and purified.

For investigation of morphological and physiological characteristics, strain CTN-1^T was cultivated on LB medium at 30 °C. Cell morphology and motility were examined by light microscopy (Olympus; ×1000) and transmission electron microscopy (H-7650; Hitachi) by using cells from an exponentially growing culture. Gram-staining was performed by using the non-staining method described by Buck (1982). The physiological properties of strain CTN-1^T were determined by using previously described tests for hydrolysis of casein, aesculin, gelatin, starch and urea (Brown, 2007), hippurate (Kinyon & Harris, 1979), elastin (Ohman *et al.*, 1980), guanine and adenine (Wallace *et al.*, 1995), cellulose (Ten *et al.*, 2004) and Tween 20 (Atlas, 1993). Catalase activity was determined with 3 % (v/v) H_2O_2, and oxidase activity was determined by using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Enzyme activity and acid production from different carbohydrates were determined by using API ZYM, API 50CH, API 20NE and API ID 32GN kits according to the manufacturer's instructions (bioMérieux). API ZYM strips were read after 5 h of incubation. The assimilation of single carbon substrates was determined by using API ID 32GN and API 20NE strips cultured at 30 °C for 24 h. Growth at 4, 10, 15, 25, 30, 37, 42 and 45 °C and at pH 4.0–10.0 (at intervals of 1.0 pH unit) was assessed after 5 days of incubation on LB agar. Growth on nutrient agar, trypticase soy agar (TSA; Difco), R2A agar (Difco) and LB agar (Difco) was also evaluated at 30 °C. Salt tolerance was tested in LN medium (LB without NaCl) supplemented with 0–5 % (w/v) NaCl after 10 days of incubation. Biodegradation of chlorothalonil was tested according to the method described by Zhang *et al.* (2007). All these physiological and biochemical tests were performed together with the reference strain, *Lysobacter daejeonensis* DSM 17634^T, which was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany).

Polar lipid and quinone analyses of strain CTN-1^T were carried out by the Identification Service of the DSMZ as described by Tindall (1990a, b). The cellular fatty acid profiles of strain CTN-1^T and *L. daejeonensis* DSM 17634^T were analysed by the China Center of Industrial Culture Collection by using a gas chromatograph, and both of these strains were cultured in R2A agar in flasks on a rotary shaker (shaking at 160 r.p.m.) at 30 °C for 48 h. The G+C content of the genomic DNA was determined by thermal denaturation (Mandel & Marmur, 1968), for which *Escherichia coli* K-12 was used as a standard. Genomic DNA from strain CTN-1^T was extracted and purified according to standard procedures (Sambrook & Russell, 2001). The 16S rRNA gene cloned into pMD-18T (Takara) was sequenced by using an automated sequencer (Applied Biosystems model 3730). The 16S rRNA gene sequence of strain CTN-1^T was compared with known sequences found in the GenBank database by using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and also identified in the EzTaxon server 2.1 (http://147.47.212.35:8080/; Chun *et al.*, 2007). Alignment of the 16S rRNA gene sequence of strain CTN-1^T and of the type strains of recognized *Lysobacter* species was computed by using the CLUSTAL X program (Thompson *et al.*, 1997), and phylogenetic analysis was performed by using MEGA 3.1 (Kumar *et al.*, 2004). An evolutionary distance matrix was calculated by using the Kimura two-parameter distance model (Kimura, 1980), and a phylogenetic tree was reconstructed with the neighbour-joining method; the robustness of the tree was examined by bootstrap analysis of 1000 replicates (Felsenstein, 1985). DNA–DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was conducted in five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the remaining three values were utilized in the calculation of hybridization values. Levels of DNA–DNA relatedness are given as means ± SD.

Cells of strain CTN-1^T were Gram-negative, non-spore-forming, non-motile (but showing gliding activity), aerobic rods 0.5–0.6 μm wide and 1.1–1.2 μm long (see Supplementary Fig. S1 in IJSEM Online). Strain CTN-1^T grew well on nutrient agar, TSA, R2A agar and LB agar. Colonies of strain CTN-1^T on LB agar were circular, smooth with entire margins, non-transparent and pale yellow after 3 days of incubation at 30 °C (Supplementary Fig. S2). Growth of strain CTN-1^T occurred in LN medium with 0–1 % (w/v) NaCl; no growth occurred in the presence of 2.0 % (w/v) NaCl. Strain CTN-1^T grew at 15–37 °C (optimal 28–30 °C) and at pH 6.0–9.0 (optimum pH 7.0–7.5). Strain CTN-1^T was capable of degrading 20 mg chlorothalonil l^{-1} to a non-detectable level within 24 h in liquid culture. However, *L. daejeonensis* DSM 17634^T and *Lysobacter niastensis* DSM 18481^T did not show chlorothalonil-degrading ability. In the API 20NE and API ID 32 GN kits, strain CTN-1^T assimilated *N*-acetylglucosamine, inositol, suberic acid, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-glucose,

Table 1. Differential phenotypic and biomedical characteristics between strain CTN-1^T and the type strains of recognized *Lysobacter* species

Strains: 1, CTN-1^T (data from this study); 2, *L. daejeonensis* DSM 17634^T (data from this study); 3, *L. soli* DCY21^T (Srinivasan *et al.*, 2010); 4, *L. concretionis* Ko07^T (Weon *et al.*, 2007); 5, *L. gummosus* LMG 8763^T (Weon *et al.*, 2007; Ten *et al.*, 2009); 6, *L. niastensis* DSM 18481^T (Weon *et al.*, 2007; Ten *et al.*, 2009); 7, *L. antibioticus* DSM 2044^T (Bae *et al.*, 2005; Ten *et al.*, 2009; Wang *et al.*, 2009); 8, *L. capsici* KCTC 22007^T (Park *et al.*, 2008; Ten *et al.*, 2009; Wang *et al.*, 2009); 9, *L. defluvii* DSM 18482^T (Yassin *et al.*, 2007; Ten *et al.*, 2009; Wang *et al.*, 2009); 10, *L. spongiicola* JCM 14760^T (Romanenko *et al.*, 2008; Ten *et al.*, 2009; Wang *et al.*, 2009); 11, *L. enzymogenes* DSM 2043^T (Bae *et al.*, 2005; Ten *et al.*, 2009; Wang *et al.*, 2009); 12, *L. brunescens* DSM 6979^T (Bae *et al.*, 2005; Ten *et al.*, 2009; Wang *et al.*, 2009); 13, *L. oryzae* DSM 21044^T (Aslam *et al.*, 2009); 14, *L. yangpyeongensis* KACC 11407^T (Aslam *et al.*, 2009; Ten *et al.*, 2009; Wang *et al.*, 2009); 15, *L. niabensis* DSM 18244^T (Weon *et al.*, 2007; Ten *et al.*, 2009; Wang *et al.*, 2009); 16, *L. ximonensis* NRRL B-51263^T (Wang *et al.*, 2009); 17, *L. koreensis* KCTC 12204^T (Lee *et al.*, 2006; Ten *et al.*, 2009); 18, *L. panaciterrae* DSM 17927^T (Ten *et al.*, 2009; Wang *et al.*, 2009). +, Positive; −, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Cell size (μm)																		
Width	0.5–0.6	0.4–0.6	0.2–0.5	0.7–1.0	0.4	0.5–0.6	0.4	0.3–0.5	ND	0.5–0.6	0.5	0.3	0.3–0.5	0.4–0.6	0.5	0.5	0.5–0.8	1.5
Length	1.1–1.2	3.0–4.0	0.6–0.9	1.0–13.0	2.0	2.0–4.0	6.5	2.0–20.0	1.0–2.0	1.3–1.5	38.0	11.0	1.8–2.0	3.0–4.0	2.0–5.0	1.0–3.0	1.5–2.0	3.0
Colony colour*	PY	Y	Y	Y	DY–PY	LB	C	YC	Y–M	Y	DY–C	BY	PY	Y	Y	M–Y	Y	C
Nitrate reduction to nitrite	+	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−
Gliding motility	+	−	+	+	+	+	+	+	ND	−	+	+	+	−	−	+	ND	+
Growth at 5 °C	−	−	+	+	−	ND	+	−	−	+	+	+	−	−	+	−	−	−
Growth at pH 4	−	−	+	−	+	+	−	−	ND	−	+/−		−	−	−	−	ND	−
Growth at pH 10	−	−	+	−	−	−	−	−	ND	−	+		+	−	−	+	ND	−
NaCl tolerance (%, w/v)	0–1	0–3	ND	ND	0–2	0–1	0–1	0–2	0–6	0–6	0–1	0–1	0	0–1	0–1	0–1	0–2	0–3
Metabolism†	A	A	A	A	F	F	F	F	ND	A	F	ND	F	A	A	A	A	A
Chlorothalonil degradation	+	−	ND	ND	ND	−‡	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Catalase	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	−
API 20NE results																		
β-Galactosidase	−	−	−	−	+	+	+	−	−	−	+	−	ND	−	−	+	−	−
Arginine dihydrolase	+	−	ND	−	−	−	−	ND	−	−	−	+	ND	−	−	−	−	−
Aesculin hydrolysis	+	+	ND	−	+	+	−	+	−	−	+	+	ND	+	−	+	+	+
D-Glucose assimilation	+	+	+	−	+	W	+	+	−	−	+	−	−	−	−	+	−	+
Maltose assimilation	−	+	+	−	+	+	+	+	+	−	+	−	−	−	−	+	−	+
D-Mannose assimilation	−	−	+	−	+	−	+	+	−	−	+	−	−	−	−	+	−	+
API ZYM results																		
N-Acetyl-β-glucosaminidase	−	+	+	−	+	−	−	−	−	−	−	−	ND	+	+	+	−	ND
α-Galactosidase	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	ND
α-Glucosidase	−	+	+	−	−	−	−	+	+	−	+	+	+	+	+	+	−	ND
β-Galactosidase (PNPG)	−	+	−	−	−	−	+	−	+	−	+	+	−	−	−	+	−	−
β-Glucosidase	−	+	+	−	−	W	+	+	−	−	+	+	+	−	−	−	−	ND
Trypsin	+	−	−	ND	+	+	−	+	ND	−	+	ND	+	+	−	ND	−	ND
DNA G+C content (mol%)	67.1	61.7	65.4	63.8	65.7	66.6	69.2	65.4	67.1	69.0	69.0	67.7	67.4	67.3	62.5	63.5	68.9	67.0

*C, Cream; M, mucoid; DY, deep yellow; LB, light beige; PY, pale yellow; Y, yellow; YC, yellow–cream; BY, brownish yellow.
†A, Aerobic; F, facultatively anaerobic.
‡Data from this study.

potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline. Differential phenotypic and biomedical characteristics between strain CTN-1^T and the type strains of recognized *Lysobacter* species are summarized in Table 1.

The major cellular fatty acids of strain CTN-1^T were iso-$C_{16:0}$ (23.0 %), iso-$C_{15:0}$ (21.4 %) and iso-$C_{17:1}\omega9c$ (15.3 %). Minor amounts of $C_{16:1}\omega7c/C_{16:1}\omega6c$ (4.5 %), iso-$C_{15:1}$ F (4.4 %), iso-$C_{11:0}$ 3-OH (3.9 %), iso-$C_{11:0}$ (3.9 %) and iso-$C_{17:0}$ (3.5 %) were also found. The detailed fatty acid profile of strain CTN-1^T is compared with those of the type strains of recognized *Lysobacter* species in Supplementary Table S1. The presence of branched fatty acids, namely iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$ and iso-$C_{17:0}$, was consistent with the placement of strain CTN-1^T within the genus *Lysobacter* (Bae *et al.*, 2005; Weon *et al.*, 2006, 2007; Romanenko *et al.*, 2008). However, differences in the fatty acid profiles were noted. The major quinone detected in strain CTN-1^T was Q-8 (99 %); Q-7 (1 %) was detected as a minor component. This quinone system is a characteristic feature of the genus *Lysobacter* (Bae *et al.*, 2005). Strain CTN-1^T contained diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as major polar lipids, as with recognized species of the genus *Lysobacter*. However, a minor amount of phosphoaminolipid instead of phosphatidyl-*N*-methylethanolamine was found for strain CTN-1^T (Supplementary Fig. S3), which might differ between species in the genus (Park *et al.*, 2008). The DNA G+C content of strain CTN-1^T was 67.1 mol%. This value is within the range reported for the genus *Lysobacter* (Christensen & Cook, 1978; Aslam *et al.*, 2009).

An almost-complete 16S rRNA gene sequence of strain CTN-1^T consisting of 1504 bp was obtained. The phylogenetic tree shows that strain CTN-1^T clusters within the genus *Lysobacter* in the class *Gammaproteobacteria* (Fig. 1). 16S rRNA gene sequence analysis revealed that strain CTN-1^T was related most closely to *L. daejeonensis* DSM 17634^T (97.1 % similarity), *Lysobacter soli* DCY21^T (95.7 %), *Lysobacter concretionis* Ko07^T (95.5 %), *Lysobacter gummosus* LMG 8763^T (95.3 %) and *L. niastensis* DSM 18481^T (95.2 %). Strain CTN-1^T showed less than 95.0 % 16S rRNA gene sequence similarity to the type strains of other *Lysobacter* species. Based on the above results, DNA–DNA hybridization experiments were carried out between strain CTN-1^T and *L. daejeonensis* DSM 17634^T. With labelled total DNA from strain CTN-1^T, the level of DNA–DNA relatedness was 34.6±3.8 %. With labelled DNA from *L. daejeonensis* DSM 17634^T, the value was 36.1±2.9 %. These values are significantly below the 70 % cut-off proposed by Wayne *et al.* (1987) for assigning strains to different genomic species.

Strain CTN-1^T could be distinguished from recognized *Lysobacter* species based on several phenotypic, biochemical and chemotaxonomic characteristics (Table 1). Notably, strain CTN-1^T was positive for arginine dihydrolase. Strain CTN-1^T differed further by its ability to degrade chlorothalonil efficiently. On the basis of the polyphasic taxonomic approach described here, we consider that strain CTN-1^T represents a novel species of the genus *Lysobacter*, for which we propose the name *Lysobacter ruishenii* sp. nov.

Description of *Lysobacter ruishenii* sp. nov.

Lysobacter ruishenii (rui.she′ni.i. N.L. gen. n. *ruishenii* of Rui-Shen, in honour of Rui-Shen Jiao, a respected Chinese microbiologist, for his enormous contributions to the development of microbiology in China).

Cells are Gram-negative, aerobic, non-spore-forming, non-motile (but show gliding activity) and rod-shaped, about 0.5–0.6 μm wide and 1.1–1.2 μm long. Colonies grown on LB agar are convex, circular, smooth, non-transparent and pale yellow after 3 days of incubation at 30 °C. Grows at 15–37 °C (optimal 28–30 °C); no growth occurs below 4 °C or above 45 °C. The pH range for growth is 6.0–9.0 (optimal pH 7.0–7.5). Growth occurs in the absence of NaCl and no growth occurs in 2.0 % (w/v) NaCl. Oxidase- and catalase-positive. Positive for reduction of nitrates to nitrites, but negative for acid production from glucose and

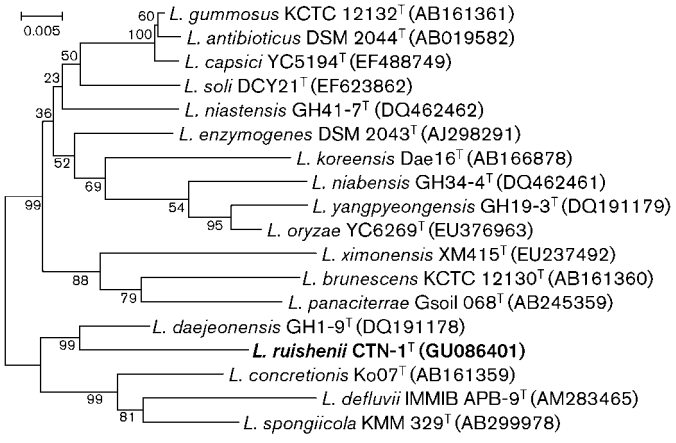

Fig. 1. Phylogenetic tree constructed by the neighbour-joining method based on 16S rRNA gene sequences of strain CTN-1^T and the type strains of recognized *Lysobacter* species. Bootstrap values expressed as percentages of 1000 replications are given at branch points. Bar, 0.005 substitutions per nucleotide position.

indole production. Hydrolyses casein, hippurate, aesculin and gelatin, but not elastin, guanine, adenine, cellulose, urea, starch or Tween 20. Utilizes *N*-acetylglucosamine, inositol, suberic acid, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-glucose, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline, but not maltose or D-mannose. Positive in tests for activities of alkaline phosphatase, esterase (C-4), esterase lipase (C-8), leucine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but not for lipase (C-14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetylglucosaminidase, α-mannosidase or α-fucosidase. Q-8 is the major quinone (99 %). The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. The main cellular fatty acids (>10 %) are iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$; minor components (<5 %) include $C_{16:1}\omega7c$/$C_{16:1}\omega6c$, iso-$C_{15:1}$ F, iso-$C_{11:0}$ 3-OH, iso-$C_{11:0}$ and iso-$C_{17:0}$. The genomic DNA G+C content of the type strain is 67.1 mol%.

The type strain, CTN-1^T (=DSM 22393^T =CGMCC 1.10136^T), was isolated from a long-term chlorothalonil-contaminated soil at the Jiangyin Suli Chemical Co., Ltd (Jiangsu, China).

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References

Ahmed, K., Chohnan, S., Ohashi, H., Hirata, T., Masaki, T. & Sakiyama, F. (2003). Purification, bacteriolytic activity, and specificity of β-lytic protease from *Lysobacter* sp. IB-9374. *J Biosci Bioeng* **95**, 27–34.

Aslam, Z., Yasir, M., Jeon, C. O. & Chung, Y. R. (2009). *Lysobacter oryzae* sp. nov., isolated from the rhizosphere of rice (*Oryza sativa* L.). *Int J Syst Evol Microbiol* **59**, 675–680.

Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.

Bae, H.-S., Im, W.-T. & Lee, S.-T. (2005). *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* **55**, 1155–1161.

Brown, A. E. (2007). *Benson's Microbiological Applications: Laboratory Manual in General Microbiology*. New York: McGraw-Hill.

Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

Caux, P., Kent, R., Fan, G. & Stephenson, G. (1996). Environmental fate and effects of chlorothalonil: a Canadian perspective. *Crit Rev Environ Sci Technol* **26**, 45–93.

Christensen, P. & Cook, F. D. (1978). *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* **28**, 367–393.

Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Cox, C. (1997). Chlorothalonil. *J Pesticide Reform* **17**, 14–20.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

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

Hashizume, H., Hattori, S., Igarashi, M. & Akamatsu, Y. (2004). Tripropeptin E, a new tripropeptin group antibiotic produced by *Lysobacter* sp. BMK333-48F3. *J Antibiot (Tokyo)* **57**, 394–399.

Islam, M. T., Hashidoko, Y., Deora, A., Ito, T. & Tahara, S. (2005). Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne peronosporomycetes. *Appl Environ Microbiol* **71**, 3786–3796.

Kazos, E. A., Nanos, C. G., Stalikas, C. D. & Konidari, C. N. (2008). Simultaneous determination of chlorothalonil and its metabolite 4-hydroxychlorothalonil in greenhouse air: dissipation process of chlorothalonil. *Chemosphere* **72**, 1413–1419.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kinyon, J. M. & Harris, D. L. (1979). *Treponema innocens*, a new species of intestinal bacteria, and emended description of the type strain of *Treponema hyodysenteriae* Harris *et al. Int J Syst Bacteriol* **29**, 102–109.

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

Lee, J. W., Im, W. T., Kim, M. K. & Yang, D. C. (2006). *Lysobacter koreensis* sp. nov., isolated from a ginseng field. *Int J Syst Evol Microbiol* **56**, 231–235.

Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.

Ohman, D. E., Cryz, S. J. & Iglewski, B. H. (1980). Isolation and characterization of *Pseudomonas aeruginosa* PAO mutant that produces altered elastase. *J Bacteriol* **142**, 836–842.

Park, J. H., Kim, R., Aslam, Z., Jeon, C. O. & Chung, Y. R. (2008). *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. *Int J Syst Evol Microbiol* **58**, 387–392.

Romanenko, L. A., Uchino, M., Tanaka, N., Frolova, G. M. & Mikhailov, V. V. (2008). *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Microbiol* **58**, 370–374.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Srinivasan, S., Kim, M. K., Sathiyaraj, G., Kim, H.-B., Kim, Y.-J. & Yang, D.-C. (2010). *Lysobacter soli* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **60**, 1543–1547.

Ten, L. N., Im, W. T., Kim, M. K., Kang, M. S. & Lee, S. T. (2004). Development of a plate technique for screening of polysaccharide-

degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.

Ten, L. N., Jung, H.-M., Im, W.-T., Yoo, S.-A., Oh, H.-M. & Lee, S.-T. (2009). *Lysobacter panaciterrae* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **59**, 958–963.

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.

Tindall, B. J. (1990a). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Tindall, B. J. (1990b). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Wallace, R. J., Jr, Brown, B. A., Blacklock, Z., Ulrich, R., Jost, K., Brown, J. M., McNeil, M. M., Onyi, G., Steingrube, V. A. & Gibson, J. (1995). New *Nocardia* taxon among isolates of *Nocardia brasiliensis* associated with invasive disease. *J Clin Microbiol* **33**, 1528–1533.

Wang, Y., Dai, J., Zhang, L., Luo, X., Li, Y., Chen, G., Tang, Y., Meng, Y. & Fang, C. (2009). *Lysobacter ximonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **59**, 786–789.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Weon, H.-Y., Kim, B.-Y., Baek, Y.-K., Yoo, S.-H., Kwon, S.-W., Stackebrandt, E. & Go, S.-J. (2006). Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils. *Int J Syst Evol Microbiol* **56**, 947–951.

Weon, H.-Y., Kim, B.-Y., Kim, M.-K., Yoo, S.-H., Kwon, S.-W., Go, S.-J. & Stackebrandt, E. (2007). *Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea. *Int J Syst Evol Microbiol* **57**, 548–551.

Yassin, A. F., Chen, W. M., Hupfer, H., Siering, C., Kroppenstedt, R. M., Arun, A. B., Lai, W. A., Shen, F. T., Rekha, P. D. & Young, C. C. (2007). *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int J Syst Evol Microbiol* **57**, 1131–1136.

Zhang, Y., Lu, J., Wu, L., Chang, A. & Frankenberger, W. T., Jr (2007). Simultaneous removal of chlorothalonil and nitrate by *Bacillus cereus* strain NS1. *Sci Total Environ* **382**, 383–387.