Lysobacter erysipheresistens sp. nov., an antagonist of powdery mildew, isolated from tobacco-cultivated soil

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A bacterial strain, RS-LYSO-3T, was isolated from tobacco-cultivated soil, collected near Chuxiong, Yunnan province, southwestern China. RS-LYSO-3T could effectively inhibit the invasion of powdery mildew on tobacco. The colonies of RS-LYSO-3T were pale yellow, and its cells were Gram-stain-negative and rod-shaped, with 68 mol% DNA G+C content. Gene sequence analysis for its 16S rRNA gene revealed the highest similarity (97.78%) with that of Lysobacter spongiicola KMM 329T. Chemotaxonomic data showed that RS-LYSO-3T possesses a quinone system with Q-8, and iso-C15 : 0 summed feature 9 and iso-C15 : 0 as the predominant fatty acids, all of which support the affiliation of RS-LYSO-3T to the genus Lysobacter. The results of DNA–DNA hybridization, physiological and biochemical tests clearly proved that RS-LYSO-3T is a representative of a novel species of the genus Lysobacter, for which the name Lysobacter erysipheresistens sp. nov. is proposed. The type strain is RS-LYSO-3T (=CCIC 23922T=JCM 31042T).

The genus Lysobacter is a member of the family Xanthomonadaceae (order Xanthomonadales, class Gammaproteobacteria), and was proposed by Christensen & Cook (1978). Some species of the genus Lysobacter were formerly classified as species of the genera Polyangium or Sorangium (Reichenbach, 2006), and include rod-shaped cells lacking fruiting bodies but with the ability to move by gliding. They possess a DNA G+C content of 62–70 mol% and form cream, brown or yellow–brown mucoid colonies. At the time of writing this manuscript, about 32 species of the genus Lysobacter had been reported from various sources such as the deep sea, soil, anaerobic reactors, ores, freshwater sediments, sewage and municipal solid waste (Bae et al., 2005; Jung et al., 2008; Lyudmila et al., 2008; Muhammad & Im, 2016; Yang et al., 2015; Yassin et al., 2007; Ye et al., 2015).

Numerous members of the genus Lysobacter show a broad spectrum of antagonism against various pathogens such as fungi, Gram-negative bacteria, Gram-positive bacteria and nematodes (Hayward et al., 2010; Ji, 2011). Therefore, species of the genus Lysobacter have a great potential in biocontrol applications.

Powdery mildew diseases are caused by a number of fungal species belonging to the order Erysiphales (Jiang, 2012). Erysiphe cichoracearum is a pathogen of tobacco (Nicotiana tabacum) and usually causes sharp deterioration in tobacco quality, usability, and even death of the entire plant.

In this study, an Erysiphe cichoracearum-resistant bacterial strain was isolated from a tobacco field near Chuxiong, Yunnan province, China. Initially, the root exudates of tobacco K326 that were sprayed with 3-acetonyl-3-hydroxyoxindole, a chemical inducer of systemic acquired resistance (Li et al., 2008), were collected as described by Geng (2011). Secondly, 5 g samples of field tobacco soil were collected and suspended in 45 ml of tobacco K326 root exudates at 30 °C for 3 days. The above soil suspension was then serially diluted 10−1–10−7 times in sterile water and was spread on modified LB agar plates (per litre of tobacco root exudates: 20 g LB broth (Beijing Aoboxing Bio-Tech)) and incubated at 30 °C for 3 days. The antagonistic effect of the isolate RS-LYSO-3T was evaluated by following the methods described in the China National Standards GB/T
**Table 1.** Differential phenotypic characteristics of RS-LYSO-3<sup>T</sup> with respect to 10 type strains of related species of the genus *Lysobacter*

Strains: 1, RS LYSO-3<sup>T</sup>; 2, *L. spingulica* KMM 329<sup>T</sup>; 3, *L. concretionis* Ko67<sup>T</sup> (data from Bae et al., 2005); 4, *L. korlensis* ZLD-17<sup>T</sup> (Zhang et al., 2011); 5, *L. Koreensis* Dae16<sup>T</sup> (Lee et al., 2006); 6, *L. arsenicirestis* ZS79<sup>T</sup> (Luo et al., 2012); 7, *L. gummous* KCTC 12132<sup>T</sup> (Park et al., 2008); 8, *L. capsici* YC5194<sup>T</sup> (Park et al., 2008); 9, *L. soli* DCY21<sup>T</sup> (Srinivasan et al., 2010); 10, *L. defluvi* IMMIB APB-9<sup>T</sup> (Yassin et al., 2007); 11, *L. antbiotics* DSM 2044<sup>T</sup> (Bae et al., 2005; Luo et al., 2012; Srinivasan et al., 2010; Yassin et al., 2007; Zhang et al., 2011). +, Positive; −, negative; w, weakly positive; ND, no data available.

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23222–2008 (Ren et al., 2009). The incidence of powdery mildew declined from 81.33±7.54% to 16.67±9.70% (mean±SEM for three independent experiments with more than 60 tobacco seedlings each).

The cell morphology of RS-LYSO-3T grown on 2216E medium (Qingdao Hope Bio-Technology) at 30°C for 4 days was observed under compound light (×1000; Olympus B-43) and scanning electron (Hitachi SU8010) microscopes.

Both RS-LYSO-3T and Lysobacter spongiicola KMM 329T, the most closely related strain, were cultured on 2216E medium. Growth at different temperatures (10, 25, 37 and 60°C) and pH (5–9) was tested. Salt tolerance was determined in 2216E medium supplemented with 0–6% NaCl (w/v). Hydrolysis of complex substrates such as adenine, xanthine, tyrosine, guanine, chitin and casein was assessed as described by Gordon (1966, 1967) and Gordon & Smith (1955). Catalase activity was examined by using 3% H2O2 (v/v). Other physiological and biochemical properties of the two strains were assessed with API 20NE, API 50CH and API ZYM kits (BioMerieux) as per the manufacturers’ instructions.

DNA was extracted with a commercial genomic DNA extraction kit (ChaoShi-Bio) and was quantified using a Nano Drop 2000c spectrophotometer (Thermo Scientific). PCR amplification was performed using the primer pair 27F and 1492R (Lane, 1991). PCR and 16S rRNA gene sequencing were performed as described by Lin et al. (2004). Editing and processing of all sequence reads was performed with the Lasergene version 7 software package using the default settings. Using the online BLAST-N (http://www.ncbi.nlm.nih.gov/BLAST/) tool, the sequences were compared against the GenBank database. Multiple alignments were performed with the program CLUSTAL X (Thompson et al., 1997). A phylogenetic tree was reconstructed using the neighbour-joining method with bootstrap analyses based on 1000 replications in the MEGA 5.0 program (Saitou & Nei, 1987). DNA–DNA hybridization was performed by the method developed by Lin (1990).

Table 1. cont.

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DNA hybridization was performed by the method developed by Lin (1990).

**Fig. 1.** Phylogenetic relationships of RS-LYSO-3T with recognized species of the genus Lysobacter and other related species. The tree was reconstructed by using the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.02 substitutions per nucleotide position.
by HPLC (SHIMADZU LC-20A) according to the method described by Xie & Yokota (2003). Whole-cell fatty acid analysis was performed according to the instructions of the Sherlock Micr biological characteristics of strain RS-LYSO-3\(^T\) with respect to 10 type strains of related species of the genus *Lysobacter*.

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*Summed feature 3 contained C\(_{16:1\!-\!7c}\) and/or C\(_{16:1\!-\!6c}\); summed feature 4 contained iso-C\(_{15:0}\)-0-2\!-\!OH and/or C\(_{16:1\!-\!7c}\); summed feature 9 contained C\(_{16:0}\)-10-methyl and/or iso-C\(_{17:0\!-\!9c}\).

Results. It only hydrolysed Tween 80 and gelatin. The physiological characteristics of strain RS-LYSO-3\(^T\) are summarized in the species description, and a comparison with the related type strains is shown in Table 1.

The 16S rRNA gene sequence of RS-LYSO-3\(^T\) is 1410 bp in length. A phylogenetic tree revealed that RS-LYSO-3\(^T\) formed a distinct subclade within the genus *Lysobacter* (Fig. 1) and its closest relative is *L. spongicola* KKM 329\(^T\) with 97.78 % sequence similarity, followed by *Lysobacter concretionis* Ko07\(^T\) (96.85 %), *L. korieensis* ZLD-17\(^T\) (96.48 %), *L. korieensis Daet\(^1\^T\) (96.62 %), *L. arsenicireisens* ZS79\(^T\) (95.99 %), *L. gummensus* KCTC 12132\(^T\) (95.56 %), *L. capsici* YC5194\(^T\) (95.49 %), *L. soli* DCY21\(^T\) (95.27 %), *L. defluvi IMIB APB-9\(^T\) (95.20 %) and *L. antbioticus* DSM 2044\(^T\) (95.19 %).

The DNA G+C content of RS-LYSO-3\(^T\) was 68.0 mol%. The respiratory quinone was Q-8 (100 %). The major
cellular fatty acids (>10%) in RS-LYSO-3T included iso-C16:0 (34.54%), summed feature 9 (13.87%) and iso-C15:0 (11.38%). Although similar fatty acids to those of type strains of members of the genus Lysobacter were found, the predominant fatty acids were different among the various species (Table 2). The major polar lipids of RS-LYSO-3T included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and unidentified phospholipids (Fig. S2).

Data on DNA G+C content, 16S rRNA gene sequence similarity, and Q-8 as the major respiratory quinone clearly indicate that RS-LYSO-3T belongs to the genus Lysobacter. However, some of its distinct physiological and biochemical characteristics are significantly different from those of the other type strains of members of the genus Lysobacter, as described in Tables 1 and 2. In addition, clear evidence is also indicated by the low DNA-DNA relatedness (43.6±9.1%, mean±SEM, N=3) between RS-LYSO-3T and its closest phylogenetic relative, L. spongicola KMM 329T. To the best of our knowledge, RS-LYSO-3T is the first member of the genus Lysobacter to show antifungal activity against Erysiphe cichoracearum. On the basis of the above evidence, RS-LYSO-3T represents a novel species of the genus Lysobacter, which is named as Lysobacter erysipheiresistens sp. nov.

**Description of Lysobacter erysipheiresistens sp. nov.**

*Lysobacter erysipheiresistens* [e.ry.si.phe.i.resis’tens. N. L. n. *erysiphe* powdery mildew (*Erysiphe cichoracearum*); L. pres. part. *resistens* resisting; N. L. part. adj. *erysipheiresistens* resisting of powdery mildew].

Cells are Gram-stain-negative, rod-shaped (0.2–0.5×1.0–2.0 μm), occurring singly or in pairs. Colonies grown on 2216E medium for 4 days are circular, pale yellow, glossy, semi-transparent and smaller than 0.5 mm in diameter. In contrast to growth inhibition on LB agar, the bacterium grows well on 2216E medium and on LB containing tobacco root exudates. Growth occurs at a range of 10–35°C, 0–5% NaCl (w/v) and pH 6–8. The major cellular fatty acids (>10%) include iso-C16:0, summed feature 9 and iso-C15:0. The bacterium hydrolyses Tween 80 and gelatin, but not starch, adenine, xanthine, tyrosine, guanine, chitin or casein. Among the 50 carbon sources tested for assimilation, RS-LYSO-3T shows positive assimilation for only D-glucose as the sole carbon source, and is weakly positive for synanthrin, phenylacetic acid, sucrose and D-galactose. RS-LYSO-3T cannot assimilate the following carbon sources: glycerol, D-ribose, β-methyl-D-xylene, D-mannose, inositol, α-methyl-D-glucoside, aesculin, lactose, glycerogen, D-lyxose, D-arabitol, 5-ketogluconate, erthritol, D-xylene, L-sorbosene, mannitol, N-acetylglucosamine, salicin, melibiose, melicetose, xylitol, D-tagatose, L-arabitol, decanoic acid, D-arabinose, L-xylene, L-rhamnose, sorbitol, amygdalin, cellobiose, raffinose, gentiobiose, D-fructose, dulcitol, α-methyl-D-mannitol, glycosides, arbutin, maltose, trehalose, starch, turanose, L-fucose, 2-ketogluconate and sodium citrate. Enzyme activities for esterase lipase (C8), cystine arylamidase, naphthol-AS-BI-phospho-hydrolase, trypsin, oxidase, alkaline phosphatase, leucine aminopeptidase and catalase are present, but urease, esterase (C4), β-glucuronidase, α-mannosidase, α-glucosidase, β-fucosidase, DNase, lipase (C14), α-galactosidase, β-glucosidase, arginine dihydrolase, β-galactosidase and N-acetylglucosaminidase activities are absent. Activities of valine arylamidase, acid phosphatase and chymotrypsin are weakly positive. Tests for nitrate reduction and indole yield negative results. Q-8 is the sole respiratory quinone.

The type strain, RS-LYSO-3T (=CCIC 23922T=JCM 31042T), was isolated from tobacco-cultivated soil at Chuxiong, Yunnan province, China. The DNA G+C content of the type strain is 68.0%.

**Acknowledgements**

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**References**


