Sphingobium scionense sp. nov., an aromatic hydrocarbon-degrading bacterium isolated from contaminated sawmill soil

Quanfeng Liang^{1,2} and Gareth Lloyd-Jones¹

Correspondence

Gareth Lloyd-Jones
gareth.lloyd-jones@
scionresearch.com

¹Scion, Te Papa Tipu Innovation Park, Private Bag 3020, Rotorua, New Zealand

²State Key Laboratory of Microbial Technology, National Glycoengineering Research Center, Shandong University, Jinan, Shandong 250100, PR China

This study characterized strain WP01^T, a Gram-staining-negative, rod-shaped, aerobic bacterium isolated from a polycyclic aromatic hydrocarbon-contaminated soil in New Zealand. Strain WP01^T shared many characteristics of the genus *Sphingobium*: the predominant respiratory quinone (89 %) was ubiquinone with ten isoprene units (Q-10); the major fatty acids were C_{18:1ω7c}, C_{16:1ω7c}, C_{16:0} and C_{14:0} 2-OH; spermidine was the major polyamine; the DNA G + C content was 63.8 mol%; and the *Sphingobium*-specific 16S rRNA signatures were conserved. A point of difference from other species of the genus *Sphingobium* was that strain WP01^T reduced nitrate to nitrite. The polar lipid pattern consisted of the predominant compounds diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipids. 16S rRNA gene sequence analysis showed that, amongst the recognized species of the genus *Sphingobium*, strain WP01^T was most similar to *Sphingobium yanoikuyae* GIFU 9882^T and *Sphingobium amiense* YT^T (>97 % 16S rRNA gene sequence similarities). The low DNA–DNA relatedness values between strain WP01^T and *S. yanoikuyae* GIFU 9882^T (46.6 %) and *S. amiense* DSM 16289^T (25.6 %) indicated no relatedness at the species level. On the basis of these characteristics, it is concluded that strain WP01^T should be considered as representing a novel species within the genus *Sphingobium*, for which the name *Sphingobium scionense* sp. nov. is proposed. The type strain is WP01^T (=DSM 19371^T=ICMP 13533^T).

Amongst the sphingomonads, many strains have been described that are able to degrade different aromatic hydrocarbons, including naphthalene and biphenyls (Lloyd-Jones & Lau, 1997; Pinyakong *et al.*, 2003; Leys *et al.*, 2004). Biphenyls and naphthalene represent two important classes of environmental pollutants, the polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Strain WP01^T was isolated previously from contaminated soil containing oil residues and *Pinus radiata* sap and sawdust and, prior to the division of the genus *Sphingomonas* by Takeuchi *et al.* (2001), had been tentatively identified as belonging to the genus *Sphingomonas* (Lloyd-Jones & Lau, 1997). In this study, a taxonomic reevaluation of strain WP01^T indicated that this strain should be regarded as a member of the genus *Sphingobium*.

The genus *Sphingomonas* was described by Yabuuchi *et al.* (1990) as comprising strictly aerobic, chemoheterotrophic, yellow-pigmented, Gram-negative, rod-shaped bacteria that

contain glycosphingolipids as cell-envelope components. Takeuchi *et al.* (1994) described four phylogenetic clusters in the genus, based on 16S rRNA gene sequence data, and subsequently combined phylogenetic, chemotaxonomic and physiological analyses to divide the genus into four genera: *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* (Takeuchi *et al.*, 2001). Recently, the genus *Sphingosinicella* (Maruyama *et al.*, 2006) has been added to the family *Sphingomonadaceae*. Here, we report on the classification of strain WP01^T as a novel member of the genus *Sphingobium*. The type species of this ever-expanding genus is *Sphingobium yanoikuyae* and species with validly published names include *S. amiense* (Ushiba *et al.*, 2003), *S. aromaticiconvertens* (Wittich *et al.*, 2007), *S. chlorophenolicum* (Takeuchi *et al.*, 2001), *S. chungbukense* (Pal *et al.*, 2005), *S. cloacae* (Prakash & Lal, 2006), *S. francense* (Pal *et al.*, 2005), *S. fuliginis* (Prakash & Lal, 2006), *S. herbicidovorans* (Takeuchi *et al.*, 2001), *S. indicum* (Pal *et al.*, 2005), *S. japonicum* (Pal *et al.*, 2005), *S. olei* (Young *et al.*, 2007), *S. xenophagum* (Pal *et al.*, 2006), *S. yanoikuyae* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 2001), *S. ummariense* (Singh & Lal, 2009) and *S. rhizovicinum* (Young *et al.*, 2008).

Abbreviation: PHA, polyhydroxyalkanoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WP01^T is EU009209.

The bacterium designated WP01^T is an aerobic, non-spore-forming, non-motile, Gram-staining-negative rod that was originally isolated for its ability to degrade phenanthrene. It is also capable of degrading naphthalene, biphenyl, *m*-xylene and benzoate (Lloyd-Jones & Lau, 1997) in mineral salts medium (Lloyd-Jones *et al.*, 1999). On nutrient agar (per litre: 3 g beef extract, 5 g peptone; Difco), yellow, round, convex and shiny colonies are formed, appearing within 2–4 days at 30 °C, which is the optimal temperature for growth. Strain WP01^T is also able to grow in Luria–Bertani (LB) broth (per litre: 10 g tryptone, 5 g yeast extract, 10 g NaCl) and is able to tolerate 0.6 M NaCl in nutrient broth. Strain WP01^T is chemoheterotrophic and is able to assimilate the carboxylic acids malate, succinate, D-gluconate and benzoate, the carbohydrates L-arabinose, D-glucose, maltose, galactose, rhamnose and xylose, and the aromatic hydrocarbons *m*-xylene, biphenyl, naphthalene and phenanthrene. Biochemical characteristics (API 20 NE; bioMérieux) include a positive catalase reaction and the abilities to hydrolyse aesculin, to produce β -galactosidase and to reduce nitrate to nitrite. Compared with *S. yanoikuyae* JCM 7371^T, with which strain WP01^T shares a similar catabolic profile, the differences are the ability of strain WP01^T to reduce nitrate and its inability to utilize citrate (Table 1). The polyhydroxyalkanoate (PHA) content and composition in biomass were determined according to the method of Riis & Mai (1988) and it was shown that strain WP01^T was able to accumulate the storage polymer PHA. The dominant ester formed by trans-esterification of the extracted PHA was propyl-3-hydroxybutyrate (identity confirmed by GC-MS analysis and by comparison with authentic standards; $99.8 \pm 0.2\%$ of the analysed esters), from which we concluded that strain WP01^T forms the aliphatic polymer poly- β -hydroxybutyrate, which, with 10 mM glucose, is accumulated to comprise up to 24.1 % of the cell-mass dry weight.

Table 1. Biochemical characteristics of strain WP01^T and the type strains of *S. amiense* and *S. yanoikuyae*

Strains: 1, WP01^T; 2, *S. amiense* YT^T; 3, *S. yanoikuyae* JCM 7371^T. All strains were positive for assimilation of D-glucose and maltose. All strains were negative for urease activity. Data for taxa 2 and 3 were taken from Ushiba *et al.* (2003).

Characteristic	1	2	3
Assimilation of:			
N-Acetyl-D-glucosamine	+	–	+
L-Arabinose	+	–	+
Citrate	–	–	+
Gluconate	+	–	+
Malate	+	–	+
D-Mannose	–	–	–
β -Galactosidase activity	+	–	+
Aesculin hydrolysis	+	–	+
Nitrate reduction	+	–	–

The cellular fatty acid composition was analysed in cells grown on trypticase soy agar at 28 °C for 24 h (MIDI). The major fatty acids of strain WP01^T were octadecenoic acid (C_{18:1} ω 7c; 56.0 %), hexadecenoic acid (C_{16:1} ω 7c; 18.8 %), hexadecanoic acid (C_{16:0}; 11.9 %) and the hydroxy fatty acid 2-hydroxytetradecanoic acid (C_{14:0} 2-OH; 9.0 %). In addition, the following minor fatty acids were identified: C_{16:1} ω 5c (1.7 %), C_{17:1} ω 7c (0.6 %), C_{16:0} 2-OH (0.7 %) and C_{18:1} ω 5c (1.3 %). The fatty acid profile of strain WP01^T contains C_{16:0}, C_{16:1}, C_{18:1} and C_{14:0} 2-OH as the major fatty acids, which are characteristic of the genus *Sphingobium* (Busse *et al.*, 1999; Yabuuchi *et al.*, 1990, 2002; Takeuchi *et al.*, 1995, 2001); however, the proportions were significantly different from those for cluster II: C_{18:1} 56.0 % versus 32 %, C_{16:1} 18.8 % versus 8 %, C_{16:0} 11.9 % versus 10 % and C_{14:0} 2-OH 9.0 % versus 34 %, for strain WP01^T and cluster II respectively (Takeuchi *et al.*, 2001).

Polar lipid and respiratory quinone analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall, DSMZ, Braunschweig, Germany. The dominant respiratory quinone was Q-10 (89 %), with lesser amounts of Q-9 (11 %). The polar lipid pattern predominately contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipid 1, with lesser amounts of sphingoglycolipid 2. Significantly smaller amounts of phosphatidylcholine and trace amounts of an unidentified aminophospholipid, which migrated close to the origin, were also present. Strain WP01^T contained the same major polar lipids as those found in *S. yanoikuyae* IFO 15102^T (Busse *et al.*, 1999); however, phosphatidyl dimethylethanolamine and an unidentified glycolipid were absent.

Polyamines were extracted and analysed by HPLC as described by Takeuchi *et al.* (2001). The polyamine pattern of strain WP01^T had spermidine as the main polyamine (1.98 μ mol g^{–1} wet weight), but homospermidine and putrescine were absent. DNA base compositions were determined by thermal denaturation (Marmur & Doty, 1962) using a spectrophotometer (DU800; Beckman) and the genomic DNA of *Escherichia coli* K-12 as the standard for the calibration of T_m values. The DNA G + C content of strain WP01^T was 63.8 mol%, which was in accordance with the range described for the genus *Sphingobium* (61.7–64.9 mol%) (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1995, 2001).

An almost full-length 16S rRNA gene sequence from strain WP01^T was obtained as described by Rochelle *et al.* (1995). Analysis of this and other published sequences revealed that strain WP01^T was most similar to bacteria previously described as belonging to *S. yanoikuyae*. Strain WP01^T shared 98 % 16S rRNA gene sequence similarity with sequences from the *S. yanoikuyae* strains Q1 (GenBank accession number U37525) and B1 (U37524.1), with which strain WP01^T also exhibited sequence similarity in the genes encoding the enzymes required for the catabolism of

aromatic compounds (Lloyd-Jones & Lau, 1997). The 16S rRNA gene sequence of strain WP01^T had high sequence similarity values with sequences from the type strains of the genus *Sphingobium*, *S. yanoikuyae* GIFU 9882^T and *S. amiense* YT^T (97.31 % and 97.16 %, respectively). The 16S rRNA gene sequence from strain WP01^T retained the nucleotide signatures that correspond to cluster II (genus *Sphingobium*) as defined by Takeuchi *et al.* (2001): 52:359 U:A, 134 G, 593 U, 987:1218 A:U and 990:1215 U:G. Phylogenetic analysis was performed by comparison with 16S rRNA gene sequences from other type strains belonging to the genus *Sphingobium*. The sequences were aligned using the CLUSTAL_X algorithm over 1335 aligned positions and a phylogenetic tree was constructed using the maximum-likelihood algorithm with 1000 bootstrap analyses using DNAMAN version 5.2.2 (Lynnon Biosoft). The results (Fig. 1) showed that strain WP01^T is related to other species of the genus *Sphingobium* and is most closely related to *S. yanoikuyae* GIFU 9882^T and *S. amiense* YT^T.

DNA–DNA hybridization was used to compare the similarity of strain WP01^T to its closest phylogenetic neighbours. Genomic DNA was extracted from strain WP01^T and *S. yanoikuyae* GIFU 9882^T and purified according to the method of Marmur (1961) with an additional proteinase K digestion and SDS treatment. DNA–DNA hybridization was carried out according to the methods of De Ley *et al.* (1970) and Huß *et al.* (1983) using a Perkin Elmer Lambda 35 UV/VIS spectrometer equipped with a PTP-6 Peltier system. The DNA–DNA relatedness value between strain WP01^T and *S. yanoikuyae* GIFU 9882^T was 46.6 %, which falls below the value of approximately 70 % that has been suggested as a threshold to delineate bacterial species (Grimont, 1999; Wayne *et al.*, 1987). Additional DNA–DNA hybridization experiments between strain WP01^T and *S. amiense* DSM 16289^T were carried out by the identification service at DSMZ. The DNA–DNA relatedness value between strain WP01^T and *S. amiense* DSM 16289^T was 25.6 %.

Strain WP01^T shares many characteristics of the genus *Sphingobium* (Takeuchi *et al.*, 2001): it is strictly aerobic and catalase-positive, has Q10 as the predominant ubiquinone and C_{18:1}ω7c as the dominant fatty acid. The DNA G+C content and 16S rRNA gene nucleotide signatures are also consistent with the genus. The ability to reduce nitrate and the low DNA–DNA relatedness values with *S. yanoikuyae* GIFU 9882^T and *S. amiense* DSM 16289^T suggest that strain WP01^T should be considered as representing a novel species within the genus *Sphingobium*, for which the name *Sphingobium scionense* sp. nov. is proposed.

Description of *Sphingobium scionense* sp. nov.

Sphingobium scionense (sci.o.nen'se. N.L. neut. adj. *scionense* pertaining to Scion, a Crown Research Institute close to the isolation source of the type strain).

Cells are Gram-negative-staining, aerobic, non-spore-forming and non-motile rods measuring 0.5–0.6 × 1.6–2.0 µm that produce yellow, round, convex and shiny colonies within 2–4 days on nutrient agar at 30 °C. Growth is observed at 15–35 °C (optimum 30 °C) but not at 37 °C. Accumulates polyhydroxyalkanoate granules in the form of poly-β-hydroxybutyrate from glucose and acetate. Positive for catalase and nitrate reductase. Negative for urease, oxidase and arginine dihydrogenase, indole production, acid production from glucose and assimilation of sorbitol, inositol, malonate, sucrose, lactose, adonitol and raffinose. The dominant fatty acids are C_{18:1}ω7c, C_{16:1}ω7c and C_{16:0} and the only hydroxy fatty acid is C_{14:0} 2-OH. The dominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipid 1, with lesser amounts of sphingoglycolipid 2. Spermidine is the main polyamine.

The type strain, WP01^T (=DSM 19371^T=ICMP 13533^T), was isolated from hydrocarbon-contaminated sawmill soil, New Zealand. The DNA G+C content of the type strain is 63.8 mol%.

Fig. 1. Phylogenetic tree based on almost-complete 16S rRNA gene sequences (1335 aligned positions) showing the relationship between strain WP01^T and type strains of species in the genus *Sphingobium*. The tree was constructed with the maximum-likelihood method. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. *Novosphingobium nitrogenifigens* Y88^T was used as the outgroup. Bar, 0.005 substitutions per nucleotide position.

Acknowledgements

This work was supported by the New Zealand Foundation for Research Science and Technology and by a Scion Postdoctoral Fellowship to Q.L. We thank Daniel van de Pas (Scion) for PHA analysis.

References

- Addison, S. L., Foote, S. M., Reid, N. M. & Lloyd-Jones, G. (2007). *Novosphingobium nitrogenifigens* sp. nov., a polyhydroxyalkanoate-accumulating diazotroph isolated from a New Zealand pulp and paper wastewater. *Int J Syst Evol Microbiol* **57**, 2467–2471.
- Busse, H.-J., Kämpfer, P. & Denner, E. B. M. (1999). Chemotaxonomic characterisation of *Sphingomonas*. *J Ind Microbiol Biotechnol* **23**, 242–251.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Grimont, P. A. D. (1999). Taxonomy and classification of bacteria. In *Manual of Clinical Microbiology*, 7th edn, pp. 249–259. Edited by P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover & R. H. Tenover. Washington, DC: American Society for Microbiology.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Leys, N. M., Ryngaert, A., Bastiaens, L., Verstraete, W., Top, E. M. & Springael, D. (2004). Occurrence and phylogenetic diversity of *Sphingomonas* strains in sites contaminated with polycyclic aromatic hydrocarbons. *Appl Environ Microbiol* **70**, 1944–1955.
- Lloyd-Jones, G. & Lau, P. C. (1997). Glutathione S-transferase-encoding gene as a potential probe for environmental bacterial isolates capable of degrading polycyclic aromatic hydrocarbons. *Appl Environ Microbiol* **63**, 3286–3290.
- Lloyd-Jones, G., Laurie, A. D., Hunter, D. W. F. & Fraser, R. (1999). Analysis of catabolic genes for naphthalene and phenanthrene degradation in contaminated New Zealand soils. *FEMS Microbiol Ecol* **29**, 69–79.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Maruyama, T., Park, H.-D., Ozawa, K., Tanaka, Y., Sumino, T., Hamana, K., Hiraishi, A. & Kato, K. (2006). *Sphingosinicella microcystinivorans* gen. nov., sp. nov., a microcystin-degrading bacterium. *Int J Syst Evol Microbiol* **56**, 85–89.
- Pal, R., Bala, S., Dadhwal, M., Kumar, M., Dhir, G., Prakash, O., Prabakaran, S. R., Shivaji, S., Cullum, J. & other authors (2005). Hexachlorocyclohexane-degrading bacterial strains *Sphingomonas paucimobilis* B90A, UT26 and Sp+, having similar *lin* genes, represent three distinct species, *Sphingobium indicum* sp. nov., *Sphingobium japonicum* sp. nov. and *Sphingobium francense* sp. nov., and reclassification of [*Sphingomonas*] *chungbukensis* as *Sphingobium chungbukense* comb. nov. *Int J Syst Evol Microbiol* **55**, 1965–1972.
- Pal, R., Bhasin, V. K. & Lal, R. (2006). Proposal to reclassify [*Sphingomonas*] *xenophaga* Stolz et al. 2000 and [*Sphingomonas*] *taejonensis* Lee et al. 2001 as *Sphingobium xenophagum* comb. nov. and *Sphingopyxis taejonensis* comb. nov., respectively. *Int J Syst Evol Microbiol* **56**, 667–670.
- Pinyakong, O., Habe, H. & Omori, T. (2003). The unique aromatic catabolic genes in sphingomonads degrading polycyclic aromatic hydrocarbons (PAHs). *J Gen Appl Microbiol* **49**, 1–19.
- Prakash, O. & Lal, R. (2006). Description of *Sphingobium fuliginis* sp. nov., a phenanthrene-degrading bacterium from a fly ash dumping site, and reclassification of *Sphingomonas cloacae* as *Sphingobium cloacae* comb. nov. *Int J Syst Evol Microbiol* **56**, 2147–2152.
- Riis, V. & Mai, W. (1988). Gas chromatographic determination of poly- β -hydroxybutyric acid in microbial biomass after hydrochloric acid propanolysis. *J Chromatogr* **445**, 285–289.
- Rochelle, P. A., Will, J. A. K., Fry, J. C., Jenkins, G. J. S., Parkes, R. J., Turley, C. M. & Weightman, A. J. (1995). Extraction and amplification of 16S rRNA genes from deep marine sediments and seawater to assess bacterial community diversity. *Nucleic Acids in the Environment*, pp. 219–239. Edited by J. T. Trevors & J. D. van Elsas. New York: Springer-Verlag.
- Singh, A. & Lal, R. (2009). *Sphingobium ummariense* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium, isolated from HCH-contaminated soil. *Int J Syst Evol Microbiol* **59**, 162–166.
- Takeuchi, M., Sawada, H., Oyaizu, H. & Yokota, A. (1994). Phylogenetic evidence for *Sphingomonas* and *Rhizomonas* as non-photosynthetic members of the alpha-4 subclass of the *Proteobacteria*. *Int J Syst Bacteriol* **44**, 308–314.
- Takeuchi, M., Sakane, T., Yanagi, M., Yamasato, K., Hamana, K. & Yokota, A. (1995). Taxonomic study of bacteria isolated from plants: proposal of *Sphingomonas rosa* sp. nov., *Sphingomonas pruni* sp. nov., *Sphingomonas asaccharolytica* sp. nov. and *Sphingomonas mali* sp. nov. *Int J Syst Bacteriol* **45**, 334–341.
- Takeuchi, M., Hamana, K. & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405–1417.
- Ushiba, Y., Takahara, Y. & Ohta, H. (2003). *Sphingobium amiense* sp. nov., a novel nonylphenol-degrading bacterium isolated from a river sediment. *Int J Syst Evol Microbiol* **53**, 2045–2048.
- 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.
- Wittich, R.-M., Busse, H.-J., Kämpfer, P., Tirola, M., Wieser, M., Macedo, A. J. & Abraham, W.-R. (2007). *Sphingobium aromaticiconvertens* sp. nov., a xenobiotic-compound-degrading bacterium from polluted river sediment. *Int J Syst Evol Microbiol* **57**, 306–310.
- Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. & Yamamoto, H. (1990). Proposals of the *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. *Microbiol Immunol* **34**, 99–119.
- Yabuuchi, E., Kosako, Y., Fujiwara, N., Naka, T., Matsunaga, I., Ogura, H. & Kobayashi, K. (2002). Emendation of the genus *Sphingomonas* Yabuuchi et al. 1990 and junior objective synonymy of the species of three genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, in conjunction with *Blastomonas ursincola*. *Int J Syst Evol Microbiol* **52**, 1485–1496.
- Young, C. C., Ho, M.-J., Arun, A. B., Chen, W.-M., Lai, W.-A., Shen, F.-T., Rekha, P. D. & Yassin, A. F. (2007). *Sphingobium olei* sp. nov., isolated from oil-contaminated soil. *Int J Syst Evol Microbiol* **57**, 2613–2617.
- Young, C.-C., Arun, A. B., Kämpfer, P., Busse, H.-J., Lai, W.-A., Chen, W.-M., Shen, F.-T. & Rekha, P. D. (2008). *Sphingobium rhizovicinum* sp. nov., isolated from rhizosphere soil of *Fortunella hindsii* (Champ. ex Benth.) Swingle. *Int J Syst Evol Microbiol* **58**, 1801–1806.