Kineococcus rhizosphaerae sp. nov., isolated from rhizosphere soil

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An orange-coloured actinomycete, designated strain RP-B16^T, was isolated from a rhizosphere soil. Cells of strain RP-B16^T were strictly aerobic, Gram-positive-staining, oxidase-negative, catalase-positive, motile cocci. A phylogenetic tree, based on 16S rRNA gene sequences, showed that members of the genus *Kineococcus* were divided into two independent clusters and that strain RP-B16^T formed a coherent clade with the type strains of *Kineococcus aurantiacus* (99.5 % sequence similarity) and *Kineococcus radiotolerans* (99.1 %). Levels of 16S rRNA gene sequence similarity between strain RP-B16^T and *Kineococcus marinus* KST3-3^T and '*Kineococcus tegulae*' T2A-S27 were lower than 97.0 %. Chemotaxonomic characteristics were typical of the genus *Kineococcus* in having meso-diaminopimelic acid as the diagnostic diamino acid, MK-9(H₂) as the predominant menaquinone, a polar lipid profile comprising diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and two unknown phospholipids, anteiso-C_{15:0} as the predominant fatty acid and a DNA G+C content of 73.8 mol%. Strain RP-B16^T showed levels of DNA–DNA relatedness of 34.6–37.3 % to *Kineococcus aurantiacus* DSM 7487^T and 31.2–32.7 % to *Kineococcus radiotolerans* DSM 14245^T. On the basis of phenotypic features and DNA–DNA hybridization data, strain RP-B16^T is considered to represent a novel species of the genus *Kineococcus*, for which the name *Kineococcus rhizosphaerae* sp. nov. is proposed. The type strain is RP-B16^T (=KCTC 19366^T =DSM 19711^T).

The genus *Kineococcus* was erected by Yokota *et al.* (1993) to encompass aerobic, Gram-positive, motile, coccoid-shaped bacteria and, at the time of writing, comprises four recognized species, namely *Kineococcus aurantiacus* (Yokota *et al.*, 1993), *Kineococcus radiotolerans* (Phillips *et al.*, 2002), *Kineococcus marinus* (Lee, 2006) and *Kineococcus gynurae* (Duangmal *et al.*, 2008). This genus, together with the genus *Kineosporia*, is phylogenetically related to members of the suborder *Frankineae* (Lee, 2006). During a study of the bacterial diversity of a cliff environment, a *Kineococcus*-like strain was isolated from a rhizosphere soil and its classification and identification were carried out by using a polyphasic approach.

Strain RP-B16^T was isolated from rhizosphere soil of a cliff-associated plant (*Peucedanum japonicum* Thunb.) on Mara Island, Jeju, Republic of Korea. Soil samples were serially diluted with sterile distilled water and aliquots (100 µl) of the dilutions were transferred onto plates of starch-casein agar. Colonies on the plates, incubated at 30 °C for 14 days, were subcultured on International *Streptomyces* Project (ISP) 2 medium (Shirling & Gottlieb, 1966) and the

pure culture was maintained in 20 % glycerol suspensions at –20 and –84 °C.

Unless specified otherwise, physiological and biochemical tests described below were performed on ISP 2 as the basal medium. Growth was tested at 0, 4, 10, 30, 37 and 42 °C and at initial pH of 4.1–12.1, as described by Lee & Lee (2007). NaCl tolerance for growth was determined in the presence of 1–10 % (w/v) NaCl (at intervals of 1 %). Gram staining, utilization of carbohydrates, hydrolysis of aesculin, casein, CM-cellulose, colloidal chitin, elastin and starch, catalase and oxidase activities and degradation of hypoxanthine, DL-tyrosine and xanthine were tested as described by Seo & Lee (2006). Anaerobic growth was tested by using a GasPak EZ Anaerobe Pouch System (Becton Dickinson) according to the manufacturer's instructions. Nitrate reduction, gelatin liquefaction and galactosidase and urease activities were examined by using the methods of MacFaddin (1980). DNA hydrolysis was tested on DNase test agar (Difco). Cell morphology and motility were observed via an Olympus light microscope equipped with phase-contrast optics (magnification × 400), with cells grown on ISP 2 medium for 3 days at 30 °C. Colony pigmentation was observed visually and was recorded after 5 days growth. Cells of strain RP-B16^T were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RP-B16^T is FM210338.

strictly aerobic, Gram-positive-staining, non-spore-forming, motile cocci and produced circular, smooth, orange colonies. Results of the physiological characterization are given in the species description and in Table 1.

Biomass for chemotaxonomic characterization was obtained from cultures grown in ISP 2 (0.4 % yeast extract, 1.0 % malt extract and 0.4 % glucose) broth for 3 days at 30 °C. Tests for the following characteristics were carried out: diagnostic peptidoglycan diamino acid (Staneck & Roberts, 1974), whole-cell sugars (Saddler *et al.*, 1991), polar lipids and respiratory quinones (Minnikin *et al.* 1977, 1984; Kroppenstedt, 1985), mycolic acids (Minnikin *et al.*, 1980) and DNA G + C content (Mesbah *et al.*, 1989). The preparation and analysis of fatty acid methyl esters were performed by GC according to the instructions of the Microbial Identification System (MIDI), with cells grown on trypticase soy agar (Difco) for 3 days at 30 °C.

The results of chemical analyses were typical for the genus *Kineococcus* (Yokota *et al.*, 1993). Whole-cell hydrolysates of strain RP-B16^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid and glucose, arabinose,

galactose, rhamnose, ribose and xylose as major sugars. Mannose was present as a minor component. *Kineococcus aurantiacus* DSM 7487^T contained glucose, rhamnose, galactose and mannose as major sugars with minor amounts of arabinose and ribose, whereas *Kineococcus radiotolerans* DSM 14245^T contained glucose and galactose as major components with minor amounts of arabinose, mannose and ribose. Xylose was not present in either of these two strains, in contrast to strain RP-B16^T. The predominant quinone was menaquinone MK-9(H₂). Major polar lipids of strain RP-B16^T were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. Additionally, two unknown phospholipids were present in trace amounts. The DNA G + C content of strain RP-B16^T was 73.8 mol%. The polar lipid profile of *Kineococcus radiotolerans* DSM 14245^T, as determined in the present study, comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and three unknown phospholipids, and its DNA G + C content was 74.3 %. The cellular fatty acid profile of strain RP-B16^T was characterized by the predominance of anteiso-C_{15:0} (72.5 % of the total). Other acids present as minor components were C_{18:0} (6.1 %),

Table 1. Differential characteristics between strain RP-B16^T and its phylogenetic relatives

Strains: 1, RP-B16^T; 2, *Kineococcus aurantiacus* DSM 7487^T (data from Yokota *et al.*, 1993 and the present study); 3, *Kineococcus gynurae* KKD096^T (Duangmal *et al.*, 2008); 4, *Kineococcus radiotolerans* DSM 14245^T (Phillips *et al.*, 2002 and the present study). +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4
Temperature for growth (°C)	10–37	9–37	14–34	11–41
pH for growth	5.1–10.1	6.1–9.1	5.0–9.0	5.1–9.1
Hydrolysis of:				
DNA	+	–	ND	+
Gelatin	+	–	+	–
Urea	+	+	+	–
Utilization of:				
D-Arabinose	–	+	–	+
Cellobiose	–	+	–	+
Inulin	+	–	–	+
Lactose	–	+	+	–
Maltose	+	–	+	–
D-Mannose	+	–	+	+
Melezitose	+	–	+	+
Methyl α-D-glucoside	–	–	ND	+
Methyl α-D-mannoside	+	–	ND	+
Raffinose	+	–	–	–
Sucrose	–	+	+	+
Trehalose	–	–	+	+
D-Xylose	–	+	–	+
Adonitol	–	w	–	+
Citrate	+	+	ND	–
Characteristic sugars*	Ara, Gal, Xyl	Ara, Gal	Ara, Gal	Ara, Gal
Polar lipids†	DPG, PG, PI, 2PL	DPG, PG, GL	ND	DPG, PG, PI, 3PL
DNA G + C content (mol%)	73.8	73.9	73.3	74.3

*Ara, Arabinose; Gal, galactose; Xyl, xylose.

†DPG, Diphosphatidylglycerol; GL, unknown glycolipid; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid.

Isolation of chromosomal DNA, amplification of the 16S rRNA gene by PCR and its sequencing and phylogenetic analyses were performed as described previously (Lee, 2006). A partial 16S rRNA gene sequence (1420 nt) for strain RP-B16^T was aligned and compared with the corresponding sequences of members of the genera *Kineococcus* and *Kineosporia* and related taxa within the suborder *Frankineae* by using the CLUSTAL X program (Thompson *et al.*, 1997). *Micromonospora chalicea* DSM 43026^T was used as an outgroup for tree construction. A neighbour-joining tree (Fig. 1) based on 16S rRNA gene sequences showed that members of the genus *Kineococcus* were divided into two independent clusters and that strain RP-B16^T formed a coherent clade with the type strains of *Kineococcus aurantiacus* (99.5 % sequence similarity), *Kineococcus radiotolerans* (99.1 %) and *Kineococcus gynurae* (96.9 %). This phylogenetic relationship was supported by a high bootstrap value of 100 % and was also found in trees constructed with other tree-making algorithms (not shown). Levels of 16S rRNA gene sequence similarity between strain RP-B16^T and *Kineococcus marinus* KST3-3^T and '*Kineococcus tegulae*' T2A-S27 were lower than 93.5 %.

On the basis of phenotypic and DNA–DNA hybridization data in addition to phylogenetic evidence, strain RP-B16^T is considered to represent a novel species of the genus *Kineococcus*, for which the name *Kineococcus rhizosphaerae* sp. nov. is proposed.

Kineococcus rhizosphaerae (rhi.zo.sphae'rae. Gr. fem. n. *rhiza* a root; L. fem. n. *sphaera* ball, sphere; N.L. gen. fem. n. *rhizosphaerae* of the rhizosphere, referring to the site from which the type strain was isolated).

The type strain, RP-B16^T (=KCTC 19366^T =DSM 19711^T), was isolated from rhizosphere soil of a cliff-associated plant (*Peucedanum japonicum* Thunb.) on Mara Island, Jeju, Republic of Korea.

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References

- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridisation from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Duangmal, K., Thamchaipenet, A., Ara, I., Matsumoto, A. & Takahashi, Y. (2008). *Kineococcus gynurae* sp. nov., isolated from a Thai medicinal plant. *Int J Syst Evol Microbiol* **58**, 2439–2442.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Huß, 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.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.
- Lee, S. D. (2006). *Kineococcus marinus* sp. nov., isolated from marine sediment of the coast of Jeju, Korea. *Int J Syst Evol Microbiol* **56**, 1279–1283.
- Lee, S. D. & Lee, D. W. (2007). *Lapillicoccus jejuensis* gen. nov., sp. nov., a novel actinobacterium of the family *Intrasporangiaceae*, isolated from stone. *Int J Syst Evol Microbiol* **57**, 2794–2798.
- MacFaddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- 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 Bacteriol* **39**, 159–167.
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr* **188**, 221–233.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Phillips, R. W., Wiegel, J., Berry, C. J., Fliermans, C., Peacock, A. D., White, D. C. & Shinkets, L. J. (2002). *Kineococcus radiotolerans* sp. nov., a radiation-resistant, Gram-positive bacterium. *Int J Syst Evol Microbiol* **52**, 933–938.
- Saddler, G. S., Tavecchia, P., Lociuero, S., Zanol, M., Colombo, E. & Selva, E. (1991). Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J Microbiol Methods* **14**, 185–191.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Seo, J. P. & Lee, S. D. (2006). *Nocardia harenae* sp. nov., an actinomycete isolated from beach sand. *Int J Syst Evol Microbiol* **56**, 2203–2207.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- 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.
- 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.
- Yokota, A., Tamura, T., Nishii, T. & Hasegawa, T. (1993). *Kineococcus aurantiacus* gen. nov., a new aerobic, gram-positive, motile coccus with meso-diaminopimelic acid and arabinogalactan in the cell wall. *Int J Syst Bacteriol* **43**, 52–57.