An aerobic, Gram-stain-positive, motile, coccus-shaped actinobacterium, designated strain L2-1-L1ᵀ, was isolated from mangrove sediment in Thailand. The organism was deep orange, oxidase-negative and catalase-positive. Growth occurred at temperatures between 17 and 32 °C and with NaCl concentrations up to 10 %. Chemotaxonomic characteristics of strain L2-1-L1ᵀ were typical of the genus *Kineococcus*. The diagnostic diamino acid of the peptidoglycan was *meso*-diaminopimelic acid. Whole-cell hydrolysates contained arabinose, galactose, glucose, mannose and ribose. The menaquinone was MK-9(H₂). Mycolic acids were not detected. Anteiso-C₁₅ : ₀ and iso-C₁₄ : ₀ were detected as the major cellular fatty acids. The major polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol and an unidentified phosphoglycolipid. The G + C content of the DNA was 74.7 mol%. A phylogenetic tree, based on 16S rRNA gene sequences, revealed that strain L2-1-L1ᵀ represents a novel member of the genus *Kineococcus*. The most closely related species were *Kineococcus endophytica* KLBMP 1274ᵀ, *Kineococcus aurantiacus* NBRC 15268ᵀ and *Kineococcus rhizosphaerae* RP-B16ᵀ, with 98.9, 98.6 and 98.5 % 16S rRNA gene sequence similarity, respectively. On the basis of phylogenetic analysis, DNA–DNA relatedness data, phenotypic characteristics and chemotaxonomic data, a novel species of the genus *Kineococcus* is proposed: *Kineococcus mangrovi* sp. nov. The type strain is strain L2-1-L1ᵀ (=BCC 75409ᵀ=NBRC 110933ᵀ).

The genus *Kineococcus* was proposed by Yokota *et al.* (1993) with *Kineococcus aurantiacus* as the type species. The genus encompasses aerobic, Gram-stain-positive, motile cocci with the presence of *meso*-diaminopimelic acid, arabinose and galactose in the cell wall. Mycolic acids are not present, and the major menaquinone is MK-9(H₂). The genus *Kineococcus* belongs to the family *Kineosporiaceae*, which accommodates the genera *Angustibacter*, *Kineococcus*, *Kineospora*, *Pseudokineococcus* and *Quadrisphaera* (Zhi *et al.*, 2009).

At the time of writing, the genus *Kineococcus* comprises seven species with validly published names (http://www.bacterio.net/), while the species *Kineococcus marinus* (Lee 2006) has been reclassified and transferred to the genus *Pseudokineococcus* (as *Pseudokineococcus marinus*) by Jurado *et al.* (2011). Members of the genus *Kineococcus* are distributed in natural environments such as plant roots and leaves (Duangmal *et al.*, 2008; Bian *et al.*, 2012), soil samples (Yokota *et al.*, 1993; Lee 2009), desert sand (Liu *et al.*, 2009), and radioactive working sites (Phillips *et al.*, 2002). Recently, a paper describing *Kineococcus gypseus* (Li *et al.*, 2015), isolated from a saline sediment, was published in the *International Journal of Systematic and Evolutionary Microbiology*.

During the investigation of actinomycetes diversity in mangrove forest at Phetchaburi province, Thailand, strain L2-1-L1ᵀ was isolated from mangrove sediment using starch casein agar (Küster & Williams, 1964) supplemented...
with antibacterial and antifungal agents (nalidixic acid 25 μg ml⁻¹ and ketokonazole 100 μg ml⁻¹). The plates were incubated at 28 °C for 14 days. The strain was isolated and purified on glucose-yeast extract agar [GYE agar; containing 1.0 % glucose (w/v), 1.0 % yeast extract (w/v) and 1.5 % agar (w/v)]. The pure culture was maintained as a 20 % glycerol suspension at −20 °C and as lyophilized cells for long-term preservation. *Kineococcus aurantiacus* JCM 10180ᵀ, *Kineococcus endophytica* KCTC 19886ᵀ and *Kineococcus rhizophaeræa* JCM 16541ᵀ were used as reference strains in this study for comparison purposes.

Cultural characteristics of strain L2-1-L1ᵀ were determined after incubation for 2 weeks at 28 °C on various media described by the International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966), Czapek's agar (Waksman, 1967), GYE agar, and nutrient agar (Waksman, 1961). The colours of colonies and soluble pigments were determined by comparing with the standard names of the 267 Colour Centroids of the NBS/ISCC Colour System (Kelly & Judd, 1976). Cell morphological characteristics of strain L2-1-L1ᵀ were observed by scanning (JSM 5600 LV; JEOL) and transmission (JEM 1220; JEOL) electron microscopy. To test motility, the strain was grown on GYE agar slants at 28 °C for 4 days before flooding with 5 ml sterile distilled water. The culture was incubated at room temperature for 10 min and cell motility was observed under a light microscope. The presence of flagella was observed by transmission electron microscopy, with cells prepared in a similar fashion to the motility test described previously; negative staining of cells was performed with 1 % uranyl acetate (Hoppert & Holzengburg, 1998). The temperature range for growth was determined on ISP medium 2. Utilization of carbohydrates as sole carbon sources was tested on ISP medium 9 (Shirling & Gottlieb, 1966). Enzyme activity profiles were tested using API ZYM system (bioMérieux) according to the manufacturer's instructions. Urease activity and hydrolysis of various substrates were evaluated using the media of Gordon et al. (1974) and Williams et al. (1983). NaCl tolerance was studied on ISP medium 2 containing NaCl at final concentration of 1–12 % (w/v), at intervals of 1 %. The ability to grow over a range of pH was studied on ISP medium 2 between pH 4–11, at intervals of 1.0 pH unit. The medium was adjusted to the appropriate pH with the following buffer systems: 0.1 M citric acid/0.1 M sodium citrate for pH 4–5; 0.1 M KH₂PO₄/0.1 M NaOH for pH 6–8; and 0.1 M NaHCO₃/0.1 M Na₂CO₃ for pH 9–11. Catalase and oxidase activities were determined with a 3 % (v/v) hydrogen peroxide solution and 1 % (w/v) tetramethyl-p-phenylenediamine solution, respectively.

Biomass used for chemotaxonomic studies was prepared by growing strain L2-1-L1ᵀ in ISP medium 2 broth incubated with shaking at 28 °C for 3 days. Cells were harvested by centrifugation and washed three times with sterile distilled water before freeze-drying. The isomers of diaminopimelic acid and sugar analysis in the whole-organism hydrolysates were determined by TLC according to the procedures described by Hasegawa et al. (1983) and Stanek & Roberts (1974). Polar lipids were extracted from freeze-dried cells and examined by two-dimensional TLC according to the procedure developed by Minnikin et al. (1977). The acyl type of the cell wall was analysed according to the method of Uchida & Aida (1984). Menaquiones were extracted and purified by the method of Collins et al. (1977), and isoprene units were subsequently analysed by LC/MS (JMS-T100LP; JEOL) with a PEGASIL ODS column (20 × 50 mm) using methanol/2-propanol (7 : 3, v/v). Mycolic acids were detected by TLC according to the method of Tomiyasu (1982). Fatty acid methyl esters were determined by GLC according to the instructions of the Sherlock Microbial Identification System (MIDI, Version 6.1) (Sasser, 1990). The resulting profiles were identified using the database library TSBA6. The G+C content (mol%) of the DNA was determined by HPLC according to the method of Tamaoka & Komagata (1984).

Genomic DNA of strain L2-1-L1ᵀ was extracted and purified following the method of Kieser et al. (2000). The 16S rRNA gene was amplified and sequenced according to the method described by Mingma et al. (2014). An almost-complete 16S rRNA gene sequence (1488 nt) was subjected to BLAST analysis and aligned with the corresponding sequences of representative genera in family *Kineosporiaceae*, retrieved from EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms drawn from the MEGA 5 software package (Tamura et al., 2011). The resultant tree topologies were evaluated by carrying out a bootstrap analysis (Felsenstein, 1985) on 1000 resampled datasets. DNA–DNA hybridization values were determined fluorometrically by the method of Ezaki et al. (1989).

Morphological observation of strain L2-1-L1ᵀ revealed that the strain showed the typical characteristics of the genus *Kineococcus*. Strain L2-1-L1ᵀ was aerobic, non-spore-forming, Gram-stain-positive, coccus-shaped and motile. The cells were 0.8–1.3 μm in diameter and occurred singly, in pairs or clusters (Fig. 1). Colonies were circular, convex and deep orange. The strain exhibited good growth on ISP medium 2 and GYE agar after 3 days incubation at 28 °C. Poor growth occurred on ISP media 3–7, Czapek's agar and nutrient agar. Melanin and diffusible pigments were not observed on any tested media. Strain L2-1-L1ᵀ was oxidase-negative and catalase-positive. The temperature range for growth was between 17 and 32 °C; no growth was observed at or above 35 °C. The strain was able to tolerate up to 10 % NaCl. Detailed physiological and biochemical properties are presented in the species description.

The almost-complete 16S rRNA gene sequence of strain L2-1-L1ᵀ (1488 nt) was compared with those of

http://ijs.microbiologyresearch.org
representatives of the family Kineosporiaceae. The phylogenetic tree indicated that isolate L2-1-L1T grouped with members of the genus Kineococcus, and formed a distinct cluster with Kineococcus endophytica KLBMP 1274T, Kineococcus aurantiacus NBRC 15268T and Kineococcus rhizosphaerae RP-B16T, supported by a bootstrap value of 91% (Fig. 2). The highest 16S rRNA gene sequence similarities were with Kineococcus endophytica KLBMP 1274T, Kineococcus aurantiacus NBRC 15268T and Kineococcus rhizosphaerae RP-B16T and were 98.9, 98.6 and 98.5%, respectively.

Strain L2-1-L1T was differentiated from the closest members of the genus Kineococcus on the basis of physiological properties in Table 1. Strain L2-1-L1T showed positive results in the utilization of L-asparagine and L-histidine, while the type strains of Kineococcus endophytica, Kineococcus aurantiacus and Kineococcus rhizosphaerae showed negative results. The type strains of Kineococcus endophytica, Kineococcus aurantiacus and Kineococcus rhizosphaerae produced trypsin and valine arylamidase, and grew at 35°C, but strain L2-1-L1T did not. Moreover, strain L2-1-L1T tolerated up to 10% NaCl, whereas the three closest related type strains were unable to do so. Chemical analysis data also indicated that the novel organism contained chemotaxonomic markers typical for members of the genus Kineococcus (Yokota et al., 1993). Strain L2-1-L1T contained meso-diaminopimelic acid in whole-cell hydrolysates and MK-9(H2) as the predominant menaquinone. The major polar lipids comprised diphasphatidylglycerol, phosphatidylglycerol and an unidentified phosphoglycolipid (Fig. S1, available in the online Supplementary Material). No mycolic acids were detected. The fatty acids profile was represented by the predominance of anteiso-C15:0 (49.6%) and iso-C14:0 (28.8%); other cellular fatty acids detected as minor components were iso-C16:1 I/C14:0 3-OH (4.9%), iso-C16:0 (3.5%), C14:0 2-OH (3.2%), C13:0 3-OH/C15:1 i H (2.8%), iso-C17:1ω5c (1.8%), C13:1 (1.1%), C16:0 (0.8%), C14:0 (0.7%), C17:0 3-OH (0.6%), iso-C15:0 (0.5%), iso-C13:0 3-OH (0.5%), C17:0 2-OH (0.4%), iso-C14:0 3-OH (0.4%) and C17:1ω7c (0.3%). The G+C content of the DNA was 74.7 mol%.

DNA–DNA relatedness studies provide a reliable way of distinguishing between representatives of species that share high 16S rRNA gene sequence similarity (Stackebrandt & Ebers, 2006). In this study, DNA–DNA hybridization values were determined with the closest phylogenetic relatives based on 16S rRNA sequences. Strain L2-1-L1T showed DNA–DNA relatedness values of 58.9%, 45.1% and 32.8% with Kineococcus endophytica KCTC 19886T, Kineococcus aurantiacus JCM 10180T and Kineococcus rhizosphaerae JCM 16541T, respectively. These values were well below 70% cut-off point recommended by Wayne et al. (1987) for assigning strains to the same species.

It is evident on the basis of genotypic and phenotypic data that strain L2-1-L1T represents a novel species of the genus Kineococcus, for which the name Kineococcus mangrovi sp. nov. is proposed.

**Description of Kineococcus mangrovi sp. nov.**


![Fig. 1. Scanning electron micrograph of strain L2-1-L1T grown on ISP medium 2 agar for 3 days at 28°C (a) and transmission electron micrograph of the strain grown on GYE agar for 4 days at 28°C (b). Bars, 10 μm (a), 500 nm (b).](image-url)
Poor growth occurs on ISP media 3, 4, 5, 6 and 7, nutrient agar and Czapek’s agar. Melanin is not detected. Nitrate reduction and urease activity are negative. Hydrogen sulphide is produced. The optimum temperature for growth is between 20 and 32 °C. Good growth occurs in ISP medium 2 containing 0–6 % NaCl, moderate growth with 8 % NaCl and poor growth with 9–10 % NaCl; no growth is observed in the presence of 11 % NaCl. Growth occurs at pH 5.0–10.0, with optimum growth at pH 5.0–8.0. Adenine, casein (skimmed milk), cellulose, gelatin, guanine, hypoxanthine, D-tyrosine, urea, xanthine and xylan are not degraded, but aesculin, arbutin, starch and Tween 80 are degraded. L-Asparagine and L-histidine are used as sole nitrogen sources, but not potassium nitrate. Cellobiose, D-fructose, D-galactose, lactose, D-mannitol, D-mannose, raffinose, D-ribose, D-sorbitol, D-xylitol, glucose, L-arabinose, L-rhamnose, maltose, melibiose, myo-inositol, sucrose, trehalose and xylose are used as sole carbon sources, but not adonitol, dextran, sodium citrate or sodium propionate. Grows in the presence of 0.005 % lysozyme. Chymotrypsin, cystine arylamidase, β-glucuronidase, lipase (C14), N-acetyl-β-glucosaminidase, trypsin and valine arylamidase activities are negative. Acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, leucine arylamidase, α-mannosidase and naphthol-AS-BI-phosphohydrolase are detected with the API ZYM enzyme assay. The diagnostic diamino acid of the peptidoglycan is meso-diaminopimelic acid. Whole-cell sugars contain arabinose, galactose, glucose, mannose and ribose. The glycan moiety of the murein is acetylated. The major

Fig. 2. Neighbour-joining tree derived from 16S rRNA gene sequences showing the relationship of strain L2-1-L1T with members of the genera Kineococcus, Kineosporia, Pseudokineococcus and Quadrisphaera. Janibacter limosus DSM 11140T was used as an outgroup taxon. Numbers at nodes indicate percentages of bootstrap support analysis of 1000 resampled datasets; only values >50 % are shown. Asterisks indicate that the clades are also recovered in maximum-likelihood and maximum-parsimony trees. Bar, 0.01 substitutions/site.
phospholipids are diphosphatidylglycerol, phosphatidylglycerol and an unidentified phosphoglycolipid. The predominant menaquinone is MK-9(H2). Mycolic acids are not detected. The major fatty acids are anteiso-C15:0 and iso-C14:0.

The type strain, strain L2-1-L1T (\(5\)BCC 75409\(5\)NBRC 110933\(5\)), was isolated from mangrove sediment, collected from Phetchaburi province, Thailand. The DNA G+C content of the type strain is 74.7 mol%.

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References


Kineococcus mangrovi sp. nov.


