Kineococcus gynurae sp. nov., isolated from a Thai medicinal plant

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A novel, Gram-positive, motile, coccus-shaped, orange-pigmented organism, designated strain KKD096T, was isolated from the roots of a Thai medicinal plant, Gynura pseudochina DC. var. hispida Thwaites. Growth of strain KKD096T occurred at temperatures of 14–34 °C, at pH 5.0–9.0 and at NaCl concentrations up to 7 % (w/v). Whole-cell hydrolysates contained arabinose and galactose as the characteristic sugars. The diagnostic diamino acid of the peptidoglycan was meso-diaminopimelic acid. The glycan moiety of the murein contained acetyl residues. The predominant menaquinone was MK-9(H2); mycolic acids were not detected. The genomic DNA G+C content was 73.3 mol%. The major cellular fatty acid was anteiso-C15 : 0 (81.42 % of the total). Strain KKD096T was assigned to the genus Kineococcus on the basis of 16S rRNA gene sequence analysis; it was most closely related to Kineococcus radiotolerans DSM 14245T (97.1 % similarity). DNA–DNA hybridization revealed 39.4 % relatedness between these two taxa. On the basis of the genotypic and phenotypic data presented, strain KKD096T is considered to represent a novel species of the genus Kineococcus, for which the name Kineococcus gynurae sp. nov. is proposed. The type strain is KKD096T (=TISTR 1856T=NRRL B-24568T=BCC 26245T=NBRC 103943T).

Abbreviation: TEM, transmission electron microscopy.

The genus Kineococcus was proposed by Yokota et al. (1993) to accommodate an aerobic, Gram-positive, motile coccus with meso-diaminopimelic acid in the whole-cell hydrolysate. At the time of writing, the genus comprises three recognized species, namely Kineococcus aurantiacus (Yokota et al., 1993), Kineococcus marinus (Lee, 2006) and Kineococcus radiotolerans (Phillips et al., 2002), which were isolated from soil in India, sediment of the coast of Korea and a radioactive environment in the USA, respectively. All members of the genus are aerobic, Gram-positive, motile, coccus-shaped bacteria.

Strain KKD096T was isolated from the roots of a Thai medicinal plant, Gynura pseudochina (L.) DC. var. hispida Thwaites, by using starch casein agar (Küster & Williams, 1964) supplemented with antibacterial and antifungal agents (nalidixic acid and ketokonazole). The plates were incubated at room temperature for 14 days. One colony was isolated and purified on glucose yeast extract (GYE) agar [1.0 % glucose (w/v), 1 % yeast extract (w/v) and 1.5 % agar (w/v)]. The pure culture was maintained as a 20 % glycerol suspension at −20 °C or as lyophilized cells for long-term preservation. Kineococcus aurantiacus IFO 15268T and Kineococcus radiotolerans DSM 14245T were used for comparative purposes.

The morphological characteristics of strain KKD096T were observed by scanning electron microscopy (JEOL-JSM 5600 LV) and transmission electron microscopy (TEM; JEM-1200EXII, JEOL).

Samples used for scanning electron microscopy and TEM were cultured for 4 days on GYE agar. Motility and production of flagella were observed by scraping cells from GYE agar plates and adding the cells to soil extract solution (5 g garden soil in 25 ml distilled water, mixed well and filtered through a 0.22 μm Millipore membrane). The cells were left at room temperature for 5–10 min before TEM observation. Negative staining of cells for TEM was performed with 1 % uranyl acetate. The ability of the novel strain to use a variety of substrates as sole carbon
sources was tested by using the API 50CH system (bioMérieux; 49 substrates). Enzyme activity profiles were tested by using the API ZYM system (bioMérieux; 19 enzyme activities tested). Urease activity was determined based on a colour change in urea broth (Gordon et al., 1974). The temperature range for growth was determined on GYE agar by using a temperature gradient incubator (Tokyo Kagaku Sangyo). Hydrolysis of casein and gelatin was evaluated by using the media of Gordon et al. (1974). Reduction of nitrate and production of melanoid pigment were determined according to the method of the International Streptomycetes Project (ISP; Shirling & Gottlieb, 1966). NaCl tolerance was studied on ISP medium 2 [containing 0.4 % yeast extract (w/v), 1 % malt extract (w/v), 0.4 % glucose (w/v) and 2 % agar (w/v)] containing NaCl at a final concentration of 1–9 % (w/v). pH tolerance was studied at pH 4–10. Catalase and oxidase activities were determined with a 3 % (v/v) hydrogen peroxide solution and 1 % tetramethyl p-phenylenediamine solution, respectively.

Cells used for chemotaxonomic analysis were obtained after incubation at 27 °C for 3 days in GYE broth. Isomers of diaminopimelic acid in the whole-organism hydrolysates were determined by TLC according to the method of Hasegawa et al. (1983). The acyl type of the cell wall was analysed according to the method of Ezaki & Aida (1984). Whole-cell sugars were analysed in accordance with Becker et al. (1965). Menaquinones were extracted and purified by using the method of Collins et al. (1977), and isoprene units were analysed by HPLC with a JASCO 802-SC chromatograph equipped with a Shiseido CAPCELL PAK C18 column (Tamaoka et al., 1983). Mycolic acids were detected by TLC according to the method of Tomiyasu (1982). The G + C content of the DNA, isolated following the method of Marmur (1961), was determined by HPLC according to Tamaoka & Komagata (1984). Analysis of the fatty acids in cells grown on ISP 2 medium at 28 °C for 72 h was performed according to the procedures for the Sherlock Microbial Identification System (Microbial ID).

PCR amplification of 16S rRNA gene samples was carried out by using standard methods (Duangmal et al., 2005). The PCR products were purified and sequenced by using an ABI PRISM Big Dye Terminator v3.0 Cycle Sequencing Ready Reaction kit (Applied Biosystems) and universal primers. The nucleotide sequences were obtained automatically by using an Applied Biosystems DNA sequencer (ABI 377) and software provided by the manufacturer. The resultant almost-complete 16S rRNA gene sequence of strain KKD096T (1351 bp) was compared via a BLAST search and aligned with the corresponding sequences of representatives of the genus Kineococcus and of related genera, retrieved from the GenBank database, by using the CLUSTAL_X (Thompson et al., 1997) and PHYDIT programs (http://plaza.snu.ac.kr/~jchun/phydit/). A phylogenetic tree was constructed by using the TREECON program (Van de Peer & De Wachter, 1994) with the Jukes & Cantor (1969) algorithm for distance estimation and the neighbour-joining method (Saitou & Nei, 1987) for tree construction. The resultant unrooted tree topologies were evaluated by carrying out a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data based on 1000 resampled datasets, by using the TREECON software. Levels of DNA–DNA relatedness were determined fluorometrically according to the method of Ezaki et al. (1989).

Cells of strain KKD096T were Gram-positive, non-spore-forming, motile, strictly aerobic cocci. Cells occurred singly, in pairs and in clusters (Fig. 1). Colonies were circular, convex and deep orange in colour on GYE agar and ISP 2 medium after 4 days at 27 °C. The results of chemical analysis indicated that strain KKD096T had chemotaxonomic markers typical of members of the genus Kineococcus (Yokota et al., 1993). Strain KKD096T contained meso-diaminopimelic acid in the peptidoglycan and MK-9(H2) as the predominant menaquinone. No mycolic acids were detected. The predominant cellular fatty acid was anteiso-C15:0 (81.42 %). The G + C content of the DNA was 73.3 mol%. Phylogenetic analysis of the almost-complete 16S rRNA gene sequence of strain KKD096T indicated that it formed a distinct cluster with members of the genus Kineococcus (except Kineococcus marinus) within the radiation encompassing members of the family Kineospiraceae (Fig. 2). The highest levels of 16S rRNA gene sequence similarity were with Kineococcus radio-tolerans DSM 14245T and Kineococcus aurantiacus IFO 15268T (97.1 and 97 %, respectively).

![Fig. 1. Scanning electron micrograph (a) and transmission electron micrograph (b) of cells of strain KKD096T grown for 4 days at 27 °C on GYE agar. Bars, 1 μm.](image-url)
Strain KKD096<sup>T</sup> was catalase- and urease-positive but oxidase-negative. The temperature range for growth was determined in a temperature gradient incubator between 5 and 50 °C. Observation of cultures over 14 days revealed growth at 14–34 °C, with the optimum temperature for growth being 25–28 °C. No growth was observed at or above 36 °C. Strain KKD096<sup>T</sup> was able to grow at pH 5.0 and 9.0, although at pH 9.0 only very poor growth was observed. Strain KKD096<sup>T</sup> was able to tolerate up to 7 % NaCl. It could be readily differentiated from recognized members of the genus *Kineococcus* on the basis of its physiological properties (Table 1).

Levels of DNA–DNA hybridization were determined from duplicate experiments with its two closest phylogenetic relatives based on 16S rRNA sequencing, namely *Kineococcus aurantiacus* IFO 15268<sup>T</sup> and *Kineococcus radiotolerans* DSM 14245<sup>T</sup> (25 and 39.4 % DNA–DNA relatedness, respectively). These results clearly indicated that strain KKD096<sup>T</sup> did not belong to *K. radiotolerans* or *K. aurantiacus* as these values were well below the recommended threshold value of 70 % for the definition of bacterial species (Wayne et al., 1987).

In conclusion, based on data from our polyphasic taxonomic study, strain KKD096<sup>T</sup> can be clearly differentiated from all recognized species of the genus *Kineococcus*. We conclude that strain KKD096<sup>T</sup> represents a novel species of the genus *Kineococcus*, for which the name *Kineococcus gynurae* sp. nov. is proposed.

**Description of *Kineococcus gynurae* sp. nov.**

*Kineococcus gynurae* [gy.nu’ra.e. N.L. n. Gynura scientific genus name of a plant; N.L. gen. n. gynurae of Gynura, isolated from *Gynura pseudochina* (L.) DC. var. hispida Thwaites, a Thai medicinal plant].

Cells are Gram-positive, aerobic, motile, non-spore-forming, catalase-positive, oxidase-negative cocci. Colonies are circular, rough and deep orange in colour. Negative for nitrate reduction but positive for urease activity. Negative for casein but positive for hydrolysis of gelatin. Growth occurs at pH 5.0–9.0 with an optimum at pH 6.0–7.0. The temperature range for growth is 14–34 °C with an optimum at 25–28 °C. In ISP 2 medium, good growth occurs at 1–5 % NaCl, moderate growth at 6 % NaCl and poor growth at 7 % NaCl.

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rose, D-sorbitol, L-sorbitose, trehalose, turanose and xyitol are utilized as sole carbon and energy sources for growth. The following substrates are not utilized: D-adenitol, amygdalin, D-arabinose, L-arabinose, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, glycogen, glycerol, inositol, inulin, methyl 2-D-glucopyranoside, methyl 2-D-mannopyranoside, N-acetylglucosamine, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, raffinose, D-ribose, salicin, starch, D-tagatose, D-xyllose and L-xyllose. Acid phosphatase, alkaline phosphatase, esterase (C4), z-galactosidase, y-glucosidase, z-glucosidase, y-glucosidase, leucine aminopeptidase, trypsin and phosphoamidase are detected with the API ZYM enzyme assay, but not chymotrypsin, cystine aminopeptidase, z-fucosidase, y-glucuronidase, lipase (C8), lipase (C14), z-mannosidase, N-acetyl-y-glucosaminidase or valine aminopeptidase. The diagnostic amino acid of the peptidoglycan is meso-diaminopimelic acid. Whole-cell sugars contain arabinose and galactose. The glycan moiety of the murein is acetylated. The predominant menaquinone is MK-9(H2). Mycolic acids are not detected. The predominant fatty acids are anteiso-C15:0 (81.42 %) and iso-C14:0 (6.34 %). Other cellular fatty acids detected as minor components are C14:0 2-0H (3.42 %), C14:0 (2.47 %), C17:0 2-0H (1.46 %) and C17:0 3-0H (1.3 %).

The type strain, KKD096T (=TISTR 1856T = NRIC B-24568 = BCC 26245T = NBRC 103943T), was isolated from the roots of Gymnura pseudochaena (L.) DC. var. hispida Thwaites, collected from Bangkok, Thailand. The DNA G+C content of the type strain is 73.3 mol%.

Acknowledgements

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References


