Streptomyces chumphonensis sp. nov., isolated from marine sediments

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Two actinomycete strains, KK1-2T and CPB4-7, were isolated from marine sediments collected in Chumphon province, Thailand. Their taxonomic positions were determined using a polyphasic approach. The morphological, cultural and chemotaxonomic characteristics of these isolates were consistent with the classification of the strains as representing a member of the genus Streptomyces. They contained LL-diaminopimelic acid in their cell wall peptidoglycan; the whole-cell sugars were ribose and glucose. The predominant menaquinones were MK9-(H6) and MK9-(H8). The major polar lipids were phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol mannosides. The predominant cellular fatty acids were anteiso-C15 : 0, iso-C16 : 0 and iso-C15 : 0. On the basis of 16S rRNA gene sequence similarity studies, these isolates were determined to be closely related to Streptomyces xinghaiensis JCM 16958T (98.2 %), Streptomyces rimosus subsp. paromomycinus JCM 4541T (98.1 %), Streptomyces sclerotialus JCM 4828T (98.1 %) and Streptomyces flocculus JCM 4476T (98.0 %). The G+C contents of the genomic DNA of strains KK1-2T and CPB4-7 were 73.3 and 74.2 mol%, respectively. They could be clearly distinguished from the related type strains by a low DNA–DNA relatedness and phenotypic differences. On the basis of these results, these strains represent a novel species of the genus Streptomyces, for which the name Streptomyces chumphonensis sp. nov. (type strain KK1-2T = JCM 18522T = TISTR 2106T = PCU 330T) is proposed.

The genus Streptomyces, the largest genus in the domain Bacteria (Hain et al., 1997), was established by Waksman & Henrici (1943) to describe the aerobic, Gram-positive, non-acid-fast bacteria which form extensively branched substrate and aerial mycelia. Species of the genus Streptomyces were classified on the basis of chemotaxonomic characteristics, 16S rRNA gene analysis and DNA–DNA relatedness (Kämpfer et al., 2008), and, at the time of writing, there are 640 recognized species (Parte, 2014). Streptomyces have attracted attention because of their ability to produce various bioactive compounds (Bérdy, 2005). The members of this genus are widely distributed in different environments, not only in soil but also in plant rhizosphere (Xiao et al., 2009), mangrove sediment (Sui et al., 2011), marine sponge (Khan et al., 2010) and marine sediment (Zhao et al., 2009). Although most of the species of the genus Streptomyces were isolated from terrestrial sources, marine species of the genus Streptomyces including ‘Streptomyces oceani’, S. axinellae, S. tateyamensis, S. marinus, S. halichlonae and S. xinghaiensis have been reported (Lam, 2006; Tian et al., 2012; Pimentel-Elardo et al., 2009; Khan et al., 2010; Zhao et al., 2009).

During our investigation of the diversity of marine actinomycetes in Thailand, we isolated two novel actinomycetes, KK1-2T and CPB4-7 from marine sediments in Chumphon province which showed the morphological characteristics of members of the genus Streptomyces. In...
In this report, we describe their isolation and taxonomic characterization based on a polyphasic approach.

Strain KK1-2T was isolated from marine sediment sample collected from Khai Island, while strain CPB4-7 was isolated from marine sediment near Chumphon beach, Chumphon Province, Thailand in June 2011. The sample was dried at room temperature for 7 days. The isolation was carried out by the standard dilution-plating technique on seawater–proline medium (proline 10.0 g, agar 15.0 g, artificial seawater 1000 ml) supplemented with cycloheximide (50 μg ml⁻¹) and nalidixic acid (25 μg ml⁻¹) and incubated at 28 °C for 2–3 weeks. A pure culture of strain KK1-2T was obtained and the isolate was maintained on yeast extract–malt extract agar (International Streptomyces Project, ISP 2 agar medium) (Shirling & Gottlieb, 1966) with seawater at 4 °C.

Morphological, cultural, physiological and biochemical characteristics of strains KK1-2T and CPB4-7 were determined by following the standard protocol of the International Streptomyces Project (ISP). All the media used in this study were prepared by using artificial seawater. Colours of the soluble pigment and the aerial and substrate mycelia were determined using the National Bureau of Standards/Inter-Society Color Council colour system. The spore morphology was observed by scanning electron microscopy (JSM-5410LV, JEOL) after cultivation on ISP2 medium at 30 °C for 14 days. Phenotypic properties were examined using standard methods (Arai, 1975; Williams & Cross, 1971). The utilization of various carbon sources was determined as described by Shirling & Gottlieb (1966). The enzyme activities were determined using the API ZYM system (bioMérieux). The effect of pH, temperature and NaCl tolerance were determined on ISP 2 medium.

Freeze-dried cells were obtained from a culture grown in ISP 2 broth on a rotary shaker at 28 °C for 5 days for chemotaxonomic analysis. The isomers of diaminopimelic acid and whole-cell sugar composition were determined according to the methods of Staneck & Roberts (1974). The acyl type of muramic acids in the cell wall was analysed by using the method of Uchida & Aida (1984). Mycolic acids were determined by TLC, using the method of Tomiyasu (1982). Polar lipids were extracted and analysed by TLC following the procedure of Minnikin et al. (1984). Isoprenoid quinones were extracted by the method of Collins et al. (1977) and were analysed by HPLC with a Cosmosil 5C18 column (4.6×150 mm, Nacalai Tesque). Fatty acid composition was analysed by using gas chromatography according to the instructions of the Microbial Identification System (MIDI) Sherlock system version 6.0 (Sassar, 1990; Kämpfer & Kropfstedt, 1996).

The genomic DNA of strain KK1-2T and CPB 4-7 were extracted from cells grown on ISP2 broth following the method of Tamaoka (1994). The DNA G+C content was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). DNA–DNA relatedness was determined according to the method of Ezaki et al. (1989). The amplification of the 16S rRNA gene were carried out as described by Suriyachakun et al. (2009) and the PCR products were sequenced (Macrogen, Seoul, Korea) by using universal primers (Lane, 1991). The 16S rRNA gene sequence was multiple-aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases by using CLUSTAL W version 1.81 (Thompson et al., 1997). Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods in the program MEGA 5.0 (Tamura et al., 2011). The confidence values of nodes were evaluated by using the bootstrap resampling method with 1000 replicates (Felsenstein, 1985).

Fig. 1. Scanning electron micrographs of strain KK1-2T grown on ISP 2 agar medium for 14 days at 30 °C. (a) Spores in straight chain; bar, 5 μm. (b) Rough surface of spores; bar, 1 μm.
as well as on nutrient agar (Table S1, available with the online Supplementary Material). Aerial mycelium and substrate mycelium were developed without fragmentation. The aerial mycelium appeared white to yellowish-white on all media. The substrate mycelium appeared light yellow on deep yellow. Both strains could grow between pH 6.0 and pH 11.0 with optimum growth at pH 8–10. The optimum growth temperature was 28–30 °C. These two strains grew in the presence of 0–10 % NaCl (w/v). Detailed physiological and biochemical properties are shown in Table 1 and in the species description.

Strains KK1-2T and CPB4-7 had identical chemotaxonomic characteristics to those of member of the genus Streptomyces. They contained LL-diaminopimelic acid in cell-wall peptidoglycan and contained mainly ribose and glucose as whole-cell hydrolysates. The N-acyl group of the muramic acid in the cell wall was the acetyl type. Mycolic acids were absent. The major polar lipids of strain KK1-2T were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol mannosides. Unknown aminolipids, unknown glycolipids, unknown nihydrin-positive glycolipid and unknown lipids were also detected (Fig. S1). This pattern corresponds to phospholipid type II of Lechevalier et al. (1977). The major menaquinones of strains KK1-2T and CPB4-7 were MK9-(H6) (68.1 and 72.4 %, respectively) and MK9-(H8) (31.9 and 27.6 %, respectively) the same as had been reported by Zhao et al. (2009). The major cellular fatty acids were anteiso-C15:0 (19.7–22.0 %), iso-C16:0 (19.9–22.7 %) and iso-C15:0 (14.2–19.0 %). Strains KK1-2T, CPB4-7 and the closely related type strains showed similar cellular fatty acid profiles but the amounts of some components were different and cyclic C17:0 was absent from the novel strains as shown in Table S2. The DNA G+C contents of strains KK1-2T and CPB 4-7 were 73.3 and 74.2 mol%, respectively.

Almost complete 16S rRNA gene sequences of strains KK1-2T (1475 nt) and CPB4-7 (1391 nt) were compared against 16S rRNA gene sequences of all members of the family Streptomycetaceae. The highest levels of 16S rRNA gene sequences similarity were with Streptomyces xinghaiensis JCM 16958T (98.2 %), Streptomyces rimosus subsp. paromomycinus JCM 4541T (98.1 %), Streptomyces sclerotialus JCM 4828T (98.1 %) and Streptomyces flocculus JCM 4476T (98.0 %). The phylogenetic tree based on 16S rRNA gene sequences of the strains KK1-2T, CPB4-7 and the most closely related type strains of species of the genus Streptomyces is shown in Fig. 2. The DNA–DNA hybridization study revealed that the labelled strain KK1-2T showed low levels of DNA–DNA relatedness to S. xinghaiensis JCM 16958T (58.3 ± 9.6 %), S. rimosus subsp. paromomycinus JCM 4541T (33.3 ± 1.4 %), S. sclerotialus JCM 4828T (48.7 ± 1.9 %) and S. flocculus JCM 4476T (55.6 ± 5.7 %). Strains KK1-2T and CPB4-7 exhibited 100 ± 0.5 % similarity to each other (Table S3). These values were lower than 70 %, the cut-off level for assigning strains to the same species (Wayne et al., 1987) and indicated that strains KK1-2T and CPB4-7 are representaties of a novel species.

In comparisons with the most closely related species with a validly published name, S. xinghaiensis JCM 16958T, our isolates could be distinguished from it by biochemical and physiological properties, in particular, gelatin liquefaction; starch hydrolysis; peptonization; growth at 45 °C; utilization of L-arabinose, fructose, myo-inositol, D-mannitol, melizitose, raffinose, sucrose and D-xylene; lipase, valine arylamidase, cystine arylamidase, acid phosphatase and N-acetyl-β-glucosaminidase (Table 1). It is evident from the phenotypic, chemotypic and genotypic data presented above that these two isolates should be classified as representing a novel species of the genus Streptomyces, for which the name Streptomyces chumphonensis sp. nov. is proposed.
Description of Streptomyces chumphonensis sp. nov.

Streptomyces chumphonensis (chum.phon.en’sis. N.L. masc. adj. chumphonensis pertaining to Chumphon province in the southern part of Thailand, where the type strain was isolated).

Aerobic, mesophilic, Gram-stain-positive actinomycetes, which produce straight, long chains of spores (Rectiflexibles) with rough surfaces and are non-motile. The spores measure 0.5–0.8–1.0 μm. White aerial spore masses and light-yellow to deep-yellow substrate mycelium are formed on all ISP media. Greyish-greenish-yellow and light olive–brown pigment are detected on ISP2 and nutrient agar, respectively. Nitrate is reduced to nitrite. Liquefaction of gelatin is variable. Peptonization is weakly positive. Hydrolysis of starch is positive but milk coagulation is negative. Optimal growth temperature is 28–30 °C; no growth occurs at 45 °C. Growth occurs at pH 6–11 and the maximum NaCl concentration for growth is 10 %.

Utilizes D-glucose but not L-arabinose, melibiose, sucrose, fructose, raffinose, D-xylene, melezitose, sorbitol, D-mannitol, myo-inositol or L-rhamnose as a sole carbon source. According to the API ZYM system, shows alkaline phosphatase, leucine arylamidase and α-glucosidase activities.

Fig. 2. Phylogenetic relationships based on neighbour-joining analysis (Saitou & Nei, 1987) of 16S rRNA gene sequences of strain KK1-2T, CPB4-7 and related species of the genus Streptomyces. Kitasatospora setae JCM 3304T was used as an outgroup. Symbols (*, #) indicate branches which were recovered in the maximum-likelihood tree and maximum-parsimony tree, respectively. Numbers at branch nodes indicate bootstrap percentages derived from 1000 replications (only values >50 % are shown). Bar, 0.005 substitutions per nucleotide position.
arylacidase, cystine arylamidase and acid phosphatase activities are weak and shows no activities of trypsin, z-chymotrypsin, naphthol-AS-BI-phosphohydrolase, z-galactosidase, z-galactosidase, z-glucuronidase, z-glucosidase, N-acetyl-z-glucosaminidase, z-mannosidase or z-fucosidase. Has z-diaminopimelic acid in the cell-wall peptidoglycan. The whole-cell sugars are glucose and ribose. No mycolic acids. The acyl type of the cell-wall muramic acid is acetyl. The major phospholipids are phosphatidylethanolamine, phosphatidylinositol, diphosphatidyglycerol, phosphatidylglycerol and phosphatidylidyinositol mannosides. Predominant menaquinones are MK9-(H6) and MK9-(H8). The type strain is JCM 18522T. The DNA G+C content of the type strain is 73.3 mol%.

The type strain, KK1-2T (=JCM 18522T=JISTR 2106T=PCR 330T) was isolated from a marine sediment collected from Khai Island, Chumphon Province, Thailand. The DNA G+C content of the type strain is 73.3 mol%.

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