Rhodococcus nanhaiensis sp. nov., an actinobacterium isolated from marine sediment

Jie Li,1 Guo-Zhen Zhao,2 Li-Juan Long,1 Fa-Zuo Wang,1 Xin-Peng Tian,1 Si Zhang1 and Wen-Jun Li2

1Key Laboratory of Marine Bio-resources Sustainable Utilization CAS, RNAM Center for Marine Microbiology, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, PR China
2Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

In this study, two strains (SCSIO 10187T and SCSIO 10197) were isolated from a sediment sample collected from the South China Sea and characterized by using a polyphasic approach. Growth was observed at 15–35 °C (optimum 28 °C) and pH 5.0–8.0 (optimum pH 6.0). Based on 16S rRNA gene sequence analysis, the strains were identified as members of the genus Rhodococcus. Phylogenetic analysis showed that the two strains clustered together and the 16S rRNA gene sequence similarities between them and other members of the genus Rhodococcus were 93.2–97.7 %. The menaquinone type was MK-8(H2). Major cellular fatty acids were C16:0, C18:1ω9c, C17:0, 10-methyl C18:0, C18:0, C19:0ω6c and C17:0ω8c. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylglycerol mannoside. The DNA G+C contents of strains SCSIO 10187T and SCSIO 10197 were 63.7 and 63.2 mol%, respectively. The combined genotypic and phenotypic data showed that the two strains represent a novel species of the genus Rhodococcus, for which the name Rhodococcus nanhaiensis is proposed; the type strain is SCSIO 10187T (=DSM 45608=CCTCC AB 2011025T), with SCSIO 10197 (=DSM 45609=CCTCC AB 2011025) as a reference strain.

The genus Rhodococcus, which belongs to the family Nocardiaceae in the phylum Actinobacteria, was described by Zopf (1891) and its description was emended by Goodfellow et al. (1998). Although a few species are pathogenic, most of them are benign and have been found to thrive in a broad range of environments, including soil, water, a grave, and plant and eukaryotic cells. Members of the genus show broad catabolic diversity and enzymic capabilities of environmental and biotechnological importance (Warhurst & Fewson, 1994; Bell et al., 1998). The genus Rhodococcus has undergone considerable expansion in the past few years and, at the time of writing, 33 species were recognized. In the present study, the taxonomic status of two isolates, SCSIO 10187T and SCSIO 10197, was determined using a polyphasic taxonomic approach. On the basis of the genotypic and phenotypic data reported here, the two isolates should be recognized as representatives of a novel species of the genus Rhodococcus.

Strains SCSIO 10187T and SCSIO 10197 were isolated from a sediment sample collected from the South China Sea (20° 44.8951’ N 114° 15.2932’ E) at a depth of 84.5 m, by the serial dilution technique using A1 medium (10 g starch, 4 g yeast extract, 2 g peptone, 1 l seawater and 12 g agar, pH 7.0) and A4 medium (0.25 g yeast extract, 0.5 g K2HPO4, 1 l seawater and 12 g agar, pH 7.0) supplemented with nalidixic acid (20 mg l−1) and cycloheximide (20 mg l−1), respectively. The purified strains were routinely cultivated on nutrient agar at 28 °C and stored as aqueous glycerol suspension (20 %, v/v) at −80 °C.

Gram staining was carried out by using the Gram stain kit (Guangdong HuanKai Microbial Sci. & Tech.) after incubation for 48 h on trypticase soy agar medium (Becton Dickinson) at 28 °C, and cell motility was confirmed by the development of turbidity throughout a tube containing semisolid medium (Leifson, 1960). Cell morphology was observed by light microscopy (Nikon Eclipse E600). Growth at different temperatures (10, 15, 20, 28, 35, 37,...
40, 45 and 50 °C) and NaCl concentrations (0, 1, 3, 5, 7, 10, 12 and 15 %, w/v) was tested on nutrient agar medium as the basal medium by incubating the cultures for 28 days. The pH range for growth was tested (at pH 4, 5, 6, 7, 8, 9 and 10, using the buffer system described by Xu et al., 2005) at 28 °C for 28 days by culturing the strains in trypticase soy broth (TSB; Becton Dickinson). Catalase activity was detected by the production of bubbles after the addition of a drop of 3 % H2O2. Oxidase activity was determined by the oxidation of tetramethyl-p-phenylenediamine. Carbon source utilization was determined according to the methods of Shirling & Gottlieb (1966) and Locci (1989). Nitrogen source utilization was assessed according to the methods of Shirling & Gottlieb (1966) and Staneck & Roberts (1974). Amino acids in the cell-wall hydrolysates was determined using TLC as described by Minnikin et al. (1977). The cell mass used for chemotaxonomic analyses was obtained from cultures grown in TSB on a rotary shaker at 28 °C, at pH 6.0 and in 3–7 % (w/v) NaCl. Both strains were positive for catalase and oxidase activities and nitrate reduction. Tests for H2S production, milk coagulation, milk peptonization, and hydrolysis of cellulose, starch, urea and gelatin were negative. Phenotypic characteristics that differentiate strains SCSIO 10187T and SCSIO 10197 from closely related type strains are given in Table 1.

The cell mass used for chemotaxonomic analyses was obtained from cultures grown in TSB on a rotary shaker at 28 °C (200 r.p.m.) for 3 days till mid-exponential growth phase. The diaminopimelic acid isomer of whole-cell hydrolysates was determined using TLC as described by Stanek & Roberts (1974). Amino acids in the cell-wall hydrolysate were analysed by precolumn derivatization with o-phthalaldehyde by HPLC (Tang et al., 2009). The sugars were detected by precolumn derivatization with 1-phenyl-3-methyl-5-pyrazoline by HPLC (Agilent 1100) according to the method described by Tang et al. (2009). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1979; Collins & Jones, 1980). Menaquinones were extracted according to Collins et al. (1977) and separated by HPLC (Tamaoka et al., 1983). Mycolic acids were extracted and analysed according to the protocol of Minnikin et al. (1980). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions. Fatty acid methyl esters were analysed by using the Microbial Identification software package (Sherlock Version 6.1; MIDI database: TSBa). The G+C content of the genomic DNA was determined by using the HPLC method (Mesbah et al., 1989).

Strains SCSIO 10187T and SCSIO 10197 had identical chemotaxonomic characteristics, which were similar to those of members of the genus Rhodococcus. Both strains contained meso-diaminopimelic acid in the cell wall. Arabinose, fucose, galactose, glucosamine, mannose and ribose were found as whole-cell sugars. Both of them contained only one menaquinone, MK-8(H2). Mycolic acids were present (Fig. S1, available in IJSEM Online). The major polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside (Fig. S2). The major cellular fatty acids (≥5.0 %) were C16:0 (26.39 %), C18:1ω9c (20.22 %), C17:0 (12.74 %), 10-methyl C18:0 (8.67 %), C18:0 (7.09 %), C19:0 (6.09 %) and C17:1ω8c (5.27 %), with smaller amounts of C19:1ω11c/ω9c (3.48 %), C16:1ω7c/ω6c (3.44 %), C14:0 (1.52 %), C20:0 (1.43 %), 10-methyl C17:0 (1.38 %), C15:0ω5c (0.29 %) and C13:0 (0.20 %), which is very similar to those described for recognized Rhodococcus species. The DNA G+C contents of strains SCSIO 10187T and SCSIO 10197 were 63.7 and 63.2 mol%, respectively.

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA genes were performed as described by Li et al. (2007). The phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon server (Chun et al., 2007). The almost-complete 16S rRNA gene sequence determined in this study was aligned with reference sequences of the genus Rhodococcus by using the program CLUSTAL_X (Thompson et al., 1997). The phylogenetic trees were constructed by the neighbour-joining (NJ; Saitou & Nei, 1987), maximum-parsimony (MP; Fitch, 1971) and maximum-likelihood (ML; Felsenstein, 1981) tree-making algorithms by using the software packages MEGA version 4.0 (Tamura et al., 2007) and PHYLIP version 3.6 (Felsenstein, 2002). Topologies of the phylogenetic trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Phylogenetic analysis revealed that strains SCSIO 10187T and SCSIO 10197 (16S rRNA gene sequence of 1485 bp and 1484 bp, respectively) were members of the family Nocardiaceae and formed a distinct lineage within the genus Rhodococcus. Strains SCSIO 10187T and SCSIO 10197 shared 99.7 % 16S rRNA gene sequence similarity and exhibited 83.0 % DNA–DNA relatedness, and thus they were considered to be members of the same genomic
Table 1. Differential physiological characteristics of *Rhodococcus nanhaiensis* strains SCSIO 10187<sup>T</sup> and SCSIO 10197 and the type strains of close phylogenetic relatives in the genus *Rhodococcus*

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species (Stackebrandt & Goebel, 1994). On the basis of 16S rRNA gene sequence comparisons, isolates SCSIO 10187<sup>T</sup> and SCSIO 10197 were most closely related to *Rhodococcus wratislaviensis* NCIMB 13082<sup>T</sup> (accession no. Z37138; 97.7% similarity), and the sequence similarities between the isolates and other members of the genus *Rhodococcus* were ranged from 93.2% (*Rhodococcus kroppenstedtii* KO7-23<sup>T</sup>) to 97.6% (*Rhodococcus opacus* DSM 43205<sup>T</sup> and *Rhodococcus marinonascens* DSM 43752<sup>T</sup>). In the NJ phylogenetic tree (Figs 1 and S3), strains SCSIO 10187<sup>T</sup> and SCSIO 10197 formed a sister lineage, which was well-separated from other members of the genus *Rhodococcus*. This relationship was also evident in the phylogenetic trees generated by the ML and MP algorithms (Figs S4 and S5).

It has been shown that some *Rhodococcus* species share high 16S rRNA gene sequence similarities within the range 98.6–99.8%, but have low DNA–DNA relatedness values (Zhang et al., 2005; Ghosh et al., 2006; Mayilraj et al., 2006; Xu et al., 2007; Li et al., 2008; Wang et al., 2010). Stackebrandt & Ebers (2006) also demonstrated that strains showing less than 98.7% 16S rRNA gene sequence similarity presented DNA reassociation values always lower than 70%. In view of the lower 16S rRNA gene sequence similarity values of strains SCSIO 10187<sup>T</sup> and SCSIO 10197 with *R. wratislaviensis* NCIMB 13082<sup>T</sup> and other close relatives, and distinguishing phenotypic traits, DNA–DNA hybridization was not investigated.

Besides the genotypic evidence, various physiological and biochemical characteristics support the distinctiveness of strains SCSIO 10187<sup>T</sup> and SCSIO 10197 from their closest relatives (Table 1). Strains SCSIO 10187<sup>T</sup> and SCSIO 10197 were unable to utilize D-glucose, whereas this ability was observed in all other strains studied. The novel isolates could also be differentiated based on the ability to degrade urea and utilization of carbon and nitrogen sources. Growth temperature and pH and tolerance to NaCl concentrations also differed, e.g. the novel isolates were unable to grow at 10 °C or 40 °C and strains SCSIO 10187<sup>T</sup> and SCSIO 10197 were moderately halophilic, which also distinguished them from close relatives. On the basis of the data described above, strains SCSIO 10187<sup>T</sup> and SCSIO 10197 represent a novel species of the genus *Rhodococcus*, for which the name *Rhodococcus nanhaiensis* sp. nov. is proposed.

**Description of Rhodococcus nanhaiensis sp. nov.**

*Rhodococcus nanhaiensis* [nan.hai.en’sis. N.L. masc. adj. nanhaiensis pertaining to Nanhai sea area (South China Sea), from where the type strain was isolated].
Gram-positive, aerobic, non-motile and mesophilic actinobacterium that shows a rod–coccus cycle during the growth phase. Colonies are creamish pink, opaque and convex with slightly irregular edges on ISP 2 agar after 7 days of incubation at 28 °C. Temperature range for growth is 15–35 °C, with optimal growth occurring at 28 °C. The pH range for growth is 5.0–8.0 (optimum pH 6.0). The NaCl concentration range for growth is 0–10% (optimum 3–7%, w/v). Positive for catalase and oxidase activities and nitrate reduction, but negative for H₂S production, milk coagulation, milk peptonization, and hydrolysis of Tween 20, 40 and 80, starch, gelatin and urea. D-Fructose and sodium pyruvate are utilized as sole carbon sources, but myo-inositol, lactose, maltose, hexadecanoate, 10-methyl-C₁₇ : 0, 10-methyl-C₁₈ : 0, 10-methyl-C₁₉ : 0 and 10-methyl-C₁₁₀₈c. The polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. The predominant menaquinones are MK-8(H₂) and mycolic acids are present. The DNA G+C content is 63.2–63.7 mol%.

The type strain, SCSIO 10187T (=DSM 45608T=CCTCC AB 2011024T), was isolated from a sediment sample collected from the South China Sea (20° 44.8951' N 114° 15.2932' E) at a depth of 84.5 m.

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