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Actinoplanes sediminis sp. nov., isolated from marine sediment

Zhi Qu,[1,2] Xiao-dong Bao,[2] Qing-yi Xie,[1,2,*] You-xing Zhao,[2] Bing Yan,[1] Hao-fu Dai[2] and Hui-Qin Chen[2]

Abstract

An actinomycete strain M4I47^T was isolated from sediment from Megas Gialos, Syros, Greece. The results of phylogenetic analysis of the 16S rRNA gene sequence of M4I47^T indicated that the highest similarity was with *Actinoplanes atraurantiacus* Y16^T (98.9 %), *Actinoplanes deccanensis* IFO 13994^T (98.8 %), *Actinoplanes digitatis* IFO 12512^T (98.1 %) and *Actinoplanes abujensis* A4029^T (98.0 %). The cell wall of the novel isolate contained *meso*-diaminopimelic acid and the whole-cell sugars were xylose, arabinose and glucose. The predominant menaquinones were MK-9(H_4), MK-9(H_6) and MK-9(H_2). The phospholipid profile comprised phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and an unknown phospholipid. The DNA G+C content was 71.5 mol%. Furthermore, a combination of DNA–DNA relatedness and some physiological and biochemical properties indicated that the novel strain could be readily distinguished from the most closely related species. On the basis of these phenotypic and genotypic data, M4I47^T represents a novel species of the genus *Actinoplanes,* for which the name *Actinoplanes sediminis* sp. nov. is proposed. The type strain is M4I47^T (=CCTCC AA 2016022^T =DSM 100965^T).

The genus *Actinoplanes*, belonging to the family *Micromonosporaceae*, was proposed by Couch [1]. The phenotypic and phylogenetic analysis of species of the genus *Actinoplanes* has been described by Goodfellow *et al.* [2] and Tamura and Hatano [3]. Members of the genus are characterized by the presence of spherical, cylindrical, digitate, lobate, bottle- or flask-shaped or very irregular sporangia [4]. Production of aerial hyphae is scant. The characteristic menaquinone of the genus is MK-9(H_4), and phosphatidylethanolamine is the diagnostic phospholipid. The genus *Actinoplanes* is one of the members of the family *Micromonosporaceae* and its members have gradually been recognized as an important source of secondary metabolites [5], such as actaplanin [6], teichomycins [7], gardimycin [8] and ramoplanin [9]. At the time of writing, the genus comprised 45 species with validly published names, including the recently described *Actinoplanes bogorensis* [10] and *Actinoplanes subglobosus* [11].

In the course of investigating actinomycete resources, strain M4I47^T was isolated from sediment from Megas Gialos, Syros, Greece (GPS: 37° 25.9′ N, 24° 56.3′ E). The resultant 10^{-1} dilution was initially ribolysed with the FastPrep-Instrument for 2 s at a speed of 4.0 m s^{-1}, and then diluted to 10^{-2} and 10^{-3}. The soil suspensions were spread over the surfaces of the agar plates of medium M4 [12], supplemented with potassium dichromate (30 ml l^{-1}), novobiocin (5 mg l^{-1}) and nystatin (30 mg l^{-1}). After 4 weeks of aerobic incubation at 28 °C, the isolate, which formed a yellowish colony, was transferred and purified on yeast extract–malt extract (International Streptomyces Project medium number 2, ISP 2) [13] agar and maintained as working cultures on ATCC 172 medium (http://www.atcc.org).

The morphological characteristics of M4I47^T were assessed by scanning electron microscopy (Evo18; Zeiss) of 28-day-old cultures on ISP 2. Cultural characteristics of M4I47^T and the reference strains were determined following growth on tap-water agar, Czapek's agar [14], GYM agar [15], ATCC172, M8 agar [16], modified Bennett's and ISP 1–7 media for 14–21 days at 28 °C. The ISCC-NBS colour charts were used to determine the names and designations of colony colors [17]. The Gram reaction was performed according to the protocol of Gregersen [18] by using KOH for cell lysis. Phenotypic characteristics of M4I47^T and its phylogenetically closest neighbours, such as temperature range (4, 10, 16, 28, 37, 40, 45, 50 and 55 °C), pH (4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11, using the buffer system described by Xie *et al.* [12] and NaCl [0–7 % (w/v), at intervals of 1 %] tolerance for growth were determined on ISP 2 for 14–21 days at 28 °C. Gelatin

Author affiliations: [1]Guangxi Mangrove Research Center, Guangxi Key Lab of Mangrove Conservation and Utilization, Beihai 536000, PR China; [2]Key Laboratory of Tropical Microbial Resources, Hainan Province, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, PR China.
***Correspondence:** Qing-yi Xie, xieqingyi@itbb.org.cn
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Abbreviations: CMC, carboxymethyl cellulose; GPS, Global Positioning System; MIDI, Microbial Identification System; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIMS, phosphatidylinositol mannosides; PL, unknown phospholipid; PME, phosphatidylmethylethanolamine.
The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of *Actinoplanes sediminis* M4I47^T is KR347172.
One supplementary table and two supplementary figures are available with the online version of this article.

liquefaction, nitrate reduction and starch hydrolysis were determined through cultivation on various media as described by Arai [19] and Williams and Cross [20]. Hydrogen sulfide production and melanoid pigment production was determined on Tresner's agar [21] and ISP 6 agar [13]. Aesculin utilization, carboxymethyl cellulose (CMC) degradation and catalase activity were determined according to the method of Trujillo *et al.* [22, 23]. Carbon source utilization was tested on basal medium [13] and each carbon source, sterilized using a 0.22 µm filter, was added to the medium aseptically to give a concentration of approximately 1 %. The utilization of amino acids as nitrogen sources was tested as described by Williams *et al.* [24]. Other physiological and biochemical characteristics of M4I47^T and the reference strains were tested by the procedures of Williams *et al.* [24] and Kämpfer *et al.* [25].

Biomass for molecular systematic analysis and freeze-dried cells used for the chemotaxonomic studies were obtained and washed after growing in ISP 2 at 28 °C for 14 days on a rotary shaker (220 r.p.m.). Cell wall amino acids and whole-cell sugars were analysed according to the procedure of Lechevalier and Lechevalier [26]. The *N*-acyl group of muramic acid in the peptidoglycan was determined by the method of Uchida and Aida [27]. The presence of mycolic acids was determined by the method of Minnikin *et al.* [28]. Polar lipids in cells were extracted and identified by the method of Minnikin *et al.* [29]. Fatty acid methyl esters were prepared by the method of Sasser [30] and the composition was determined using the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were identified by using the Microbial Identification software package (Sherlock Version 6.0; MIDI database: ACTIN6). Menaquinones were extracted according to the protocol of Minnikin *et al.* [29] and finally analysed using an established HPLC procedure [31].

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out as described by Nakajima *et al.* [32]. The PCR products were blunt-end ligated to the plasmid vector pEGM-T (Promega), the ligation products were transformed into competent *Escherichia coli* DH5α cells and positive clones were sent to Shanghai South Gene Technology (Shanhai, PR China) for sequencing using T 7 and Sp 6 primers (Shanghai Sangon). The 16S rRNA gene sequence of M4I47^T was identified using the EzBioCloud server (https://www.ezbiocloud.net) [33] and aligned with the 16S rRNA gene sequences of other species of the genus *Actinoplanes* using CLUSTAL X version 2.1 [34]. Phylogenetic analyses were performed using the software package MEGA version 6 [35] and tree topologies were inferred using the maximum-parsimony [36], maximum-likelihood [37] and neighbor-joining methods [38]. Phylogenetic distances were calculated with Kimura's two-parameter model [39] and the stability of the tree topology was evaluated by bootstrap analysis [40] based on 1000 resamplings. Three type strains of species of the genus *Actinocatenispora* were used as outgroups. The values for sequence similarity among the most closely related 50 strains were determined using the EzBioCloud [33].

Genomic DNA of M4I47^T was extracted as described by Pospiech and Neumann [41]. The DNA G+C content of M4I47^T was determined by the HPLC method [42]. The level of DNA relatedness between M4I47^T and the related strains were measured on nylon membranes using the method described by Wang *et al.* [31].

The almost-complete 16S rRNA gene sequence (1492 nt) of M4I47^T showed a close relationship with those of members of the family *Micromonosporaceae*. Comparison of 16S rRNA gene sequences of M4I47^T and other members of the genus *Actinoplanes* revealed sequence similarities ranging from 95.8 % to 98.9 %, the species most closely related to the novel isolate were *Actinoplanes atraurantiacus* Y16^T (98.9 %), *Actinoplanes deccanensis* IFO 13994^T (98.8 %), *Actinoplanes digitatis* IFO 12512^T (98.1 %) and *Actinoplanes abujensis* A4029^T (98.0 %). The phylogenetic trees based on 16S rRNA gene sequences indicated that M4I47^T formed a distinct branch with *Actinoplanes atraurantiacus* Y16^T [4] and *Actinoplanes deccanensis* IFO 13994^T [43] by all of the tree-making algorithms with a 85 % bootstrap value (Fig. 1). Further study revealed that the DNA relatedness values between M4I47^T and *Actinoplanes atraurantiacus* Y16^T and *Actinoplanes deccanensis* IFO 13994^T, were 40.6±2.3 and 35.2±5.5 %, respectively, both of which were below the 70 % threshold value proposed by Wayne *et al.* [44], the key marker for the identification of a novel species.

The morphological and chemical properties of M4I47^T are consistent with its classification as representing a member of the genus *Actinoplanes*. M4I47^T produced well-developed and branched substrate hyphae on ISP 2 medium, approximately 0.6 µm in diameter, but no aerial hyphae (Fig. S1, available in the online version of this article). Irregular sporangia were observed on substrate hyphae, motile spores were borne singly from the substrate mycelium, with a diameter of approximately 0.8 µm. Good growth was observed on ISP 2, ISP 6, ATCC 172, M 8, GYM and Czapek's agar; moderate growth was observed on ISP 1, ISP 4, ISP 7 and modified Bennett's agar; poor growth was observed on ISP 3, ISP 5 and tap water agar. The colour of colonies on different media was deep orange yellow (ISP 2, ISP 6, ATCC 172 and M 8), vivid orange yellow (ISP 3, ISP5, ISP 7 and tap water agar), yellowish white (ISP 1 and ISP 4), brownish orange (Czapek's), moderate reddish brown (GYM) or brilliant orange yellow (modified Bennett's). Soluble pigment was produced on ISP 2, ISP 6, ATCC 172, GYM and M 8 (Table S1).

The cell wall of the novel isolate contained *meso*-diaminopimelic acid and the whole-cell sugars were xylose, arabinose and glucose. The acyl type of the cell wall was glycolyl. Mycolic acids were not detected. The characteristic polar lipids were phosphatidylethanolamine (PE), phosphatidylmethylethanolamine (PME), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIMS) and an unknown

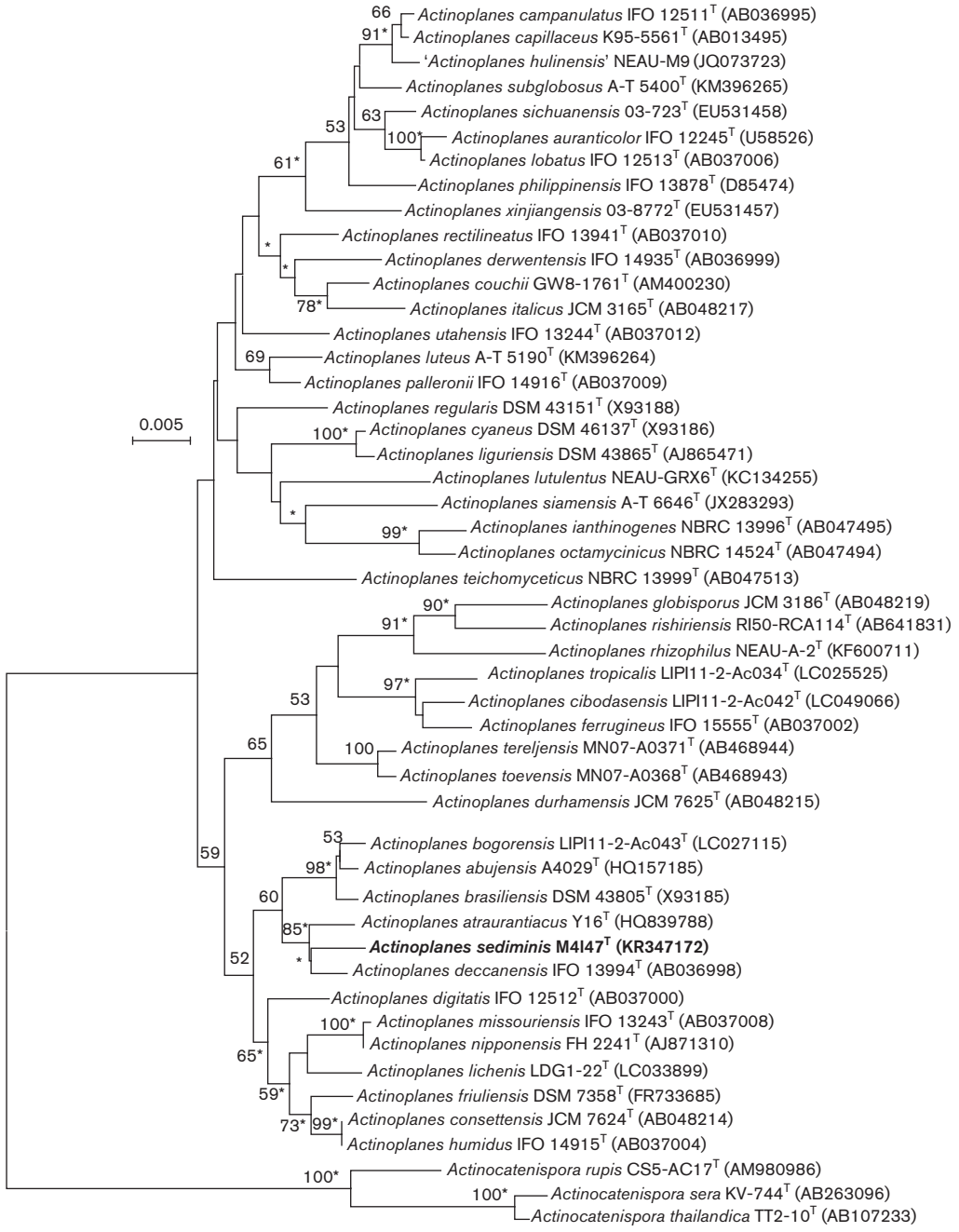

Fig. 1. Neighbour-joining phylogenetic tree [38], based on almost-complete 16S rRNA gene sequences (1400 nt), showing the relationships between M4I47^T and phylogenetically related species of the genus *Actinoplanes*. Three type strains of species of the genus *Actinocatenispora* were used as outgroups. Asterisks (*) indicate branches of the tree that were also found using the maximum-parsimony [36] and maximum-likelihood [37] methods. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates); only values exceeding 50 % are indicated. Bar, 0.005 substitutions per nucleotide position.

phospholipid (PL) (Fig. S2), corresponding to phospholipid type PII of Lechevalier *et al.* [45]. The predominant menaquinones were MK-9(H_4) (57.6 %), MK-9(H_6) (21.5 %) and MK-9(H_2) (20.9 %). The fatty acid profile of M4I47^T was composed of $C_{17:0}$ (31.8 %), anteiso-$C_{16:0}$ (16.2 %), anteiso-$C_{17:0}$ (10.9 %), iso-$C_{16:0}$ (8.1 %), anteiso-$C_{15:0}$ (5.9 %), 10-methyl-$C_{16:0}$ (5.8 %), 9-methyl-$C_{14:0}$ (5.4 %), iso-$C_{15:0}$ (4.7 %), $C_{15:0}$ (3.5 %), $C_{17:1}\omega10c$ (2.0 %), $C_{14:0}$ (1.9 %), iso-$C_{17:0}$ (1.7 %), $C_{16:0}$ (1.1 %) and $C_{16:1}\omega9c$ (0.9 %). The G+C content of the DNA was 71.5 mol%.

M4I47^T could be distinguished from its closest phylogenetic relatives of the genus *Actinoplanes* using a combination of phenotypic properties (Table 1). The results of phylogenetic analysis of the 16S rRNA gene and chemotaxonomic analyses indicated that M4I47^T represented a member of the genus *Actinoplanes*. Furthermore, a low level of DNA–DNA relatedness (35.2 and 40.6 %) was observed between M4I47^T and its closest phylogenetic relatives. It is evident from the genotypic and phenotypic data presented above that M4I47^T is distinguishable from previously described species of the genus *Actinoplanes*. Therefore, M4I47^T represents a novel species of the genus *Actinoplanes* for which the name *Actinoplanes sediminis* sp. nov. is proposed.

DESCRIPTION OF *ACTINOPLANES SEDIMINIS* SP. NOV.

Actinoplanes sediminis (se.di′mi.nis. L. gen. n. *sediminis* of sediment).

Aerobic, Gram-reaction-positive, mesophilic actinomycete that forms well-developed and branched substrate hyphae. Colonies are yellowish white to deep orange yellow. Single spores are formed on substrate hyphae. Aerial hyphae are absent. Soluble pigment is produced on ISP 2, ISP 6, ATCC 172 and M 8 media. Growth occurs at 16–37 °C (optimum, 28 °C), at pH 5.5–9.0 (optimum, pH 7.0). The maximum NaCl concentration for growth is 4 %. Nitrate is reduced to nitrite. Positive for starch hydrolysis, urea hydrolysis, melanin formation and H_2S production, but negative for gelatin liquefaction, aesculin hydrolysis and cellulose decomposition. Utilizes D-glucose, L-arabinose, cellobiose, D-fructose, raffinose, salicin, sucrose, maltose and lactose but does not utilize D-xylose, D-mannitol, L-rhamnose. D-mannose, D-galactose, riffinose, inositol or L-sorbose, as sole carbon sources. Utilizes glycine, L-arginine, L-asparagine, L-histidine and L-lysine but not L-tyrosine and L-phenylalanine as sole nitrogen sources. The cell wall contains *meso*-DAP. The characteristic whole-cell sugars are xylose, arabinose and glucose. The polar lipid profile comprises phosphatidylethanolamine (PE), phosphatidylmethylethanolamine (PME), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIMS) and an unknown phospholipid (PL). The characteristic menaquinones are MK-9(H_4), MK-9(H_2) and MK-9 (H_6). Major fatty acids are $C_{17:0}$, anteiso-$C_{16:0}$ and anteiso-$C_{17:0}$.

The type strain M4I47^T (=CCTCC AA 2016022^T=DSM 100965^T), was isolated from a sediment sample collected in Megas Gialos, Syros, Greece. The G+C content of the DNA of type strain is 71.5 mol%.

Table 1. Differential characteristics of M4I47^T and its closest relatives

Strains: 1, M4I47^T; 2, *A. atraurantiacus* Y16^T; 3, *A. deccanensis* IFO 13994^T. +, Positive; –, negative; W, weak. All strains degraded starch, formed melanin, reduced nitrate and utilized D-glucose, D-fructose, salicin, sucrose, maltose, lactose, glycine and L-histidine but did not utilize riffinose and L-phenylalanine, liquefy gelatin or decompose aesculin and cellulose. All data were obtained from this study.

Characteristic	1	2	3
Biochemical tests:			
Urea	+	+	–
H_2S	+	–	–
Cellulose decomposition	–	–	+
Growth on sole carbon sources:			
D(+)-galactose	–	+	+
Cellobiose	+	+	–
D(+)-mannose	–	+	W
Inositol	–	–	+
D(+)-xylose	–	+	–
L-arabinose	+	–	+
D-mannitol	–	W	+
Raffinose	+	–	–
L(+)-rhamnose	–	+	+
Growth on sole carbon and nitrogen sources:			
L-tyrosine	–	–	+
L-arginine	+	–	–
L-asparagine	+	+	–
L-lysine	+	–	–
Tolerance tests:			
NaCl range (%, w/v)	0–4	0–3	0–3
pH range	5.5–9.0	6.0–9.0	6.0–8.5
Temperature range	16–37 °C	16–40 °C	16–37 °C

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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