Amycolatopsis marina sp. nov., an actinomycete isolated from an ocean sediment

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A Gram-positive, aerobic, non-motile actinobacterium, designated strain Ms392AT, was isolated from an ocean-sediment sample collected from the South China Sea. The isolate contained chemical markers that supported chemotaxonomic assignment to the genus Amycolatopsis. On the basis of an analysis of 16S rRNA gene sequence similarities, strain Ms392AT represents a novel subclade within the genus Amycolatopsis, with Amycolatopsis palatopharyngis 1BDZT as its closest phylogenetic neighbour (99.4 % similarity). However, DNA–DNA hybridization demonstrated that strain Ms392AT was distinct from A. palatopharyngis AS 4.1729T (48.6 % relatedness). The polyphasic analysis demonstrated that the ocean isolate can be clearly distinguished from recognized species of the genus Amycolatopsis. Therefore, strain Ms392AT represents a novel species of the genus Amycolatopsis, for which the name Amycolatopsis marina sp. nov. is proposed. The type strain is Ms392AT (=CGMCC 4.3568T =NBRC 104263T).

The genus Amycolatopsis, classified as belonging to the family Pseudonocardiaceae (Embley et al., 1988; Warwick et al., 1994), was proposed by Lechevalier et al. (1986) for aerobic, amycolate, nocardioform actinomycetes and is well defined as a result of chemotaxonomic characterization (Lechevalier et al., 1986; Henssen et al., 1987; Mertz & Yao, 1993; Yassin et al., 1993) and phylogenetic analyses based on the comparison of 16S rRNA gene sequences (Embley et al., 1988; Warwick et al., 1994). The members of the genus Amycolatopsis are Gram-positive, non-acid-fast, non-motile actinomycetes that form branched vegetative hyphae that undergo fragmentation into rod-like and squarish elements. In addition, they are represented chemotaxonomically by the following features: wall chemotype IV (meso-diaminopimelic acid, arabinose and galactose in cell-wall hydrolysates), a tetrahydrogenated menaquinone with nine isoprene units [MK-9(H4)] as the major menaquinone, a phospholipid pattern of type II sensu Lechevalier et al. (1977) (phosphatidylethanolamine as a diagnostic phospholipid), fatty acid profiles that include complex mixtures of saturated and branched-chain acids and the absence of mycolic acids. The members of this genus have DNA G+C contents in the range 66–73 mol%.

At the time of writing, the genus Amycolatopsis comprises 38 recognized species, most of which were isolated from various terrestrial environments (Goodfellow et al., 2001; Kim et al., 2002; Saintpierre-Bonaccio et al., 2005; Lee et al., 2006; Tan et al., 2006a; Groth et al., 2007; Carlsohn et al., 2007) or clinical material (Labeleda et al., 2003; Huang et al., 2004). On the basis of chemotaxonomic and morphological markers (Kim & Goodfellow, 1999) and analyses using genus-specific oligonucleotide primers (Tan et al., 2006b), Amycolatopsis strains were separated from members of the other genera classified within the family Pseudonocardiaceae. Recently, there has been an explosion of information about novel bioactive compounds isolated from members of the genus Amycolatopsis (Demain & Zhang, 2005; Zhang et al., 2005). In an effort to explore the
relatively untapped potential of members of this genus and investigate potential applications for their secondary metabolites (Zhang et al., 2007), we attempted to isolate and identify strains from the South China Sea.

Strain Ms392AT was isolated using the following procedure. Fresh deep-ocean sediment samples were collected in the South China Sea and kept at 4 °C for isolation as soon as possible. Serial dilutions of sample suspensions were transferred onto the selective isolation medium (SM1; Tan et al., 2006b) for the genus Amycolatopsis and incubated at 28 °C for 4 weeks.

Strain Ms392AT was cultivated on ISP 2, ISP 3, ISP 4 and ISP 5 media (Shirling & Gottlieb, 1966) at 28 °C. Spore chains were observed for colonies grown using the coverslip technique of Kawato & Shinobu (1959). Morphological characteristics were examined by using light microscopy (CX41; Olympus) and scanning electron microscopy (S-570; Hitachi). Morphological features were observed on ISP 2 and ISP 4 media at 28 °C. The phenotypic properties of the isolate were consistent with its classification within the genus Amycolatopsis. The whitish aerial mycelium, which was produced only on ISP 2 and ISP 4, formed rod-like mycelial fragments. No diffusion pigments were produced on any of the media tested.

Physiological and biochemical characteristics of strain Ms398AT are given in Table 1 and in the species description. Carbohydrate utilization was tested using ISP 9 (Shirling & Gottlieb, 1966) as the basal medium with filter-sterilized compounds at a final concentration of 1% (w/v). Urease activity was determined by checking for a colour change in Bacto urea broth (Difco). The production of H2S was tested on peptone iron agar (Difco). Nitrate reductase activity was determined using a 1% (w/v) solution of tetramethylphenylenediamine (Kovács, 1956).

The procedures used for the identification of cell-wall amino acids and sugars in whole-cell hydrolysates were those described by Stanek & Roberts (1974). Menaquinones were extracted by using the method of Collins et al. (1977) and were analysed by means of HPLC, as described by Tamaoka et al. (1983). Polar lipids were extracted as described by Minnikin et al. (1979) and identified by using two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Biomass for quantitative fatty acid analysis of strain Ms398AT was prepared by scraping growth from TSB agar plates that had been incubated for 7 days at 28 °C. Fatty acids were extracted, methylated and analysed using the MIDI (Microbial Identification) system. The cell-wall diaminopimelic acid in the peptidoglycan layer of strain Ms392AT was meso-diaminopimelic acid, the major sugars in the cell wall were arabinose and galactose (cell-wall chemotype IV) according to Lechevalier & Lechevalier (1980) and the predominant isoprenoid quinones were tetrahydrogenated menaquinones with eight and nine isoprene units. The phospholipids included diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylylserine and glycerophosphorylethanolamine. The major fatty acids were C16.0 (40.35%) and C16.1 (11.42%). Others fatty acids that occurred in smaller amounts were C16.1 cis9 (8.61%), C16.1 cis9 (7.79%), C16.0 (7.08%), C17.0 (4.89%), iso-C17.0 (3.46%), C15.0 (2.91%), iso-C16.0 H (2.66%), anteiso-C17.0 (2.28%), C15.1 B (1.63%), iso-C15.0 (1.50%), 10-methyl C16.0 (1.39%), C18.0 (8.61%).

Table 1. Differential phenotypic characteristics of strain Ms392AT and its closest phylogenetic neighbour, A. palatopharyngis AS 4.1729T

Data were taken from this study or from Huang et al., 2004.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. marina sp. nov. Ms392AT</th>
<th>A. palatopharyngis AS 4.1729T</th>
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<tbody>
<tr>
<td>Utilization as sole carbon source of:</td>
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<tr>
<td>(+)-L-Arabinose</td>
<td>–</td>
<td>w</td>
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<tr>
<td>(+)-L-Rhamnose</td>
<td>+</td>
<td>–</td>
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<td>(+)-Maltose</td>
<td>+</td>
<td>–</td>
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<tr>
<td>(+)-Raffinose</td>
<td>–</td>
<td>w</td>
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<tr>
<td>Decomposition of:</td>
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<tr>
<td>L-Lysine</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>W</td>
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<td>Production of:</td>
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<tr>
<td>Catalase</td>
<td>+</td>
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<td>Urease</td>
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<td>W</td>
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<td>Nitrate reductase</td>
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<td>Drug susceptibility/resistance</td>
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<td>Cephalothin</td>
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<td>R</td>
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<tr>
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<tr>
<td>pH 10.0</td>
<td>–</td>
<td>W</td>
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</table>
(1.35%), C₁₈:₁ cis9 (0.97%), iso-C₁₄:₀ (0.90%) and iso-C₁₈:₀ (0.81).

Extraction of genomic DNA and PCR amplification and 16S rRNA gene sequencing were carried out as described previously (Li et al., 2007). An almost-complete 16S rRNA gene sequence (1485 bp) was generated for the novel isolate. Preliminary comparison of this sequence against those in the GenBank database indicated that the novel isolate was closely related to the members of the family Pseudonocardiaecae. A phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA, version 3.1 (Kumar et al., 2004), after multiple alignment of the data using CLUSTAL_X (Thompson et al., 1997). Distances (using distance options according to Kimura’s two-parameter model; Kimura, 1980, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis (based on 1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985).

The phylogenetic analysis (Fig. 1) indicated that isolate Ms392AT formed a distinct clade within the radiation encompassing the members of the genus Amycolatopsis, and revealed that the isolate was most closely associated with Amycolatopsis palatopharyngis 1BDZᵀ within a separate cluster. The 16S rRNA gene sequence similarities between strain Ms392Aᵀ and Amycolatopsis species with validly published names were below 97.0%, except for A. palatopharyngis 1BDZᵀ, which showed 99.4% similarity (corresponding to 9 differences over 1427 locations).

To determine whether strain Ms392Aᵀ represents a distinct species of the genus Amycolatopsis, DNA–DNA hybridizations were performed by applying the method of He et al. (2005) with five replications for each sample. Strain Ms392Aᵀ displayed low DNA–DNA reassociation with A. palatopharyngis AS 4.1729ᵀ (mean value 48.6%). The result is far below the cut-off point recommended for the circumscription of bacterial genomic species by Wayne et al. (1987). The G+C content of the DNA was determined by using the HPLC method (Mesbah et al., 1989) and a mean value of 70.1 mol% was obtained.

Features that serve to differentiate strain Ms392Aᵀ from its closest phylogenetic neighbour, A. palatopharyngis AS 4.1729ᵀ, are shown in Table 1. Thus, in conclusion, genotypic, chemotaxonomic and phenotypic data demonstrate that strain Ms392Aᵀ represents a novel species of the genus Amycolatopsis, for which the name Amycolatopsis marina sp. nov. is proposed.

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the position of strain Ms392Aᵀ within the radiation of the genus Amycolatopsis. Bootstrap percentages (based on 1000 resamplings) are shown at nodes. Bar, 0.02 substitutions per nucleotide position.

Amycolatopsis marina sp. nov.

http://ijs.sgmjournals.org 479
Description of Amycolatopsis marina sp. nov.

Amycolatopsis marina (ma.ri‘na. L. fem. adj. marina of the sea, marine).

Cells are Gram-positive, aerobic and non-motile and produce white aerial mycelium sparsely on ISP 2 agar medium. The branched yellow to yellow–brown substrate mycelium fragments into rod-like elements. No diffusion of nutrients across the agar medium. The branched yellow to yellow–brown substrate pigments are produced on any of the media tested.

Catalase-positive and oxidase-negative. Negative for urease activity. Not L-tyrosine or casein. Cell-wall hydrolysates contain gelatin, hypoxanthine, xanthine, allantoin and starch, but not amphenicol, midecamycin, minocycline, cephalothin and chloramphenicol. (+)-D-Fructose, (+)-D-galactose, (+)-cellobiose, myo-inositol, (+)-L-rhamnose salicin, (+)-maltose, (+)-D-mannitol and (+)-trehalose are utilized as carbon sources, but sorbitol, (+)-D-lactose, dextrin, arabinose, (+)-raffinose and (-)-sucrose are not. Decomposes gelatin, hypoxanthine, xanthine, allantoin and starch, but not l-tyrosine or casein. Cell-wall hydrolysates contain meso-diaminopimelic acid, arabinose and galactose. MK-9(H2) (79 %) is the predominant menaquinone; MK-8(H2) (13 %) is also present. The phospholipids comprise diphasatidylglycerol, phosphatidylmethyl ethanolamine, phosphatidylethanolamine, phosphatidylinositol, phosphaticidylglycerol and phosphatidylinositol mannolide. The major cellular fatty acids are iso-C16:0 and iso-C16:0 2-0H. The DNA G+C content of the type strain is 70.1 mol%.

The type strain, Ms392AT (=CGMCC 4.3568T =NBRC 104263T), was isolated from an ocean-sediment sample collected in the South China Sea.

Acknowledgements

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References


