Actinomycetospora rhizophila sp. nov., an actinomycete isolated from rhizosphere soil of a peace lily (Spathiphyllum Kochii)

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A novel actinomycete, designated strain NEAU-B-8T, was isolated from the rhizosphere soil of a peace lily (Spathiphyllum Kochii) collected from Heilongjiang province, north-east China. Key morphological and physiological characteristics as well as chemotaxonomic features of strain NEAU-B-8T were congruent with the description of the genus Actinomycetospora, such as the major fatty acids, the whole-cell hydrolysates, the predominant menaquinone and the phospholipid profile. The 16S rRNA gene sequence analysis revealed that strain NEAU-B-8T shared the highest sequence similarities with Actinomycetospora lutea JCM 17982T (99.3 % 16S rRNA gene sequence similarity), Actinomycetospora chlora TT07l-57T (98.4 %), Actinomycetospora straminea IY07-55T (98.3 %) and Actinomycetospora chibensis TT04-21T (98.2 %); similarities to type strains of other species of this genus were lower than 98 %. The phylogenetic tree based on 16S rRNA gene sequences showed that strain NEAU-B-8T formed a distinct branch with A. lutea JCM 17982T that was supported by a high bootstrap value of 97 % in the neighbour-joining tree and was also recovered with the maximum-likelihood algorithm. However, the DNA–DNA relatedness between strain NEAU-B-8T and A. lutea JCM 17982T was found to be 50.6 ± 1.2 %. Meanwhile, strain NEAU-B-8T differs from other most closely related strains in phenotypic properties, such as maximum NaCl tolerance, hydrolysis of aesculin and decomposition of urea. On the basis of the morphological, physiological, chemotaxonomic, phylogenetic and DNA–DNA hybridization data, we conclude that strain NEAU-B-8T represents a novel species of the genus Actinomycetospora, named Actinomycetospora rhizophila sp. nov. The type strain is NEAU-B-8T. (=CGMCC 4.7134T=DSM 46673T).

The genus Actinomycetospora was first established by Jiang et al. (2008) and its description was later emended by Tamura et al. (2011b) and Zhang et al. (2014). At the time of writing, the genus encompasses 11 species with validly published names (http://www.bacterio.net/actinomycetospora.html), including the recently described Actinomycetospora atypica (Zhang et al., 2014). Species of the genus Actinomycetospora have distinct features, such as being Gram-stain-positive, aerobic and non-motile and form bud-like spore chains. The cell wall contains meso-diaminopimelic acid, arabinose and galactose. The predominant menaquinone is MK-8(H4). The major fatty acid is iso-C16:0 and the diagnostic phospholipids are phosphatidylcholine and phosphatidylethanolamine. During the investigation of microbial diversity in the peace lily (Spathiphyllum Kochii) rhizosphere, strain NEAU-B-8T was isolated. We performed a taxonomic study using a polyphasic approach on this strain, and concluded that the strain represents a novel species of the genus Actinomycetospora.

Strain NEAU-B-8T was isolated from rhizosphere soil of a peace lily (Spathiphyllum Kochii) grown in a flowerpot in Heilongjiang province, north-east China (45° 45’ N 126° 41’ E). The soil sample was air-dried for 14 days at room temperature, suspended in distilled water followed by standard serial dilution in water and spread onto hemic acid-vitamin agar (HV; Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l−1) and nalidixic acid.
(50 mg l⁻¹). After 14 days aerobic incubation at 28 °C, the isolate was purified and maintained as a working culture on International Streptomyces Project (ISP) medium 3 (Shirling & Gottlieb, 1966). The type strain of Actinomycetospora lutea was purchased from the Japan Collection of Microorganisms (JCM) and cultured under the same conditions for comparative analysis.

Cultural characteristics were determined after 14 days at 28 °C by methods using ISP media 2–7 (Shirling & Gottlieb, 1966), nutrient agar (Waksman, 1961), Maltose Bennett’s agar (yeast extract 1 g; beef extract 1 g; NZ amine type A 2 g; maltose 10 g; distilled water 1 l; agar 20 g, pH 7.3) and DSMZ medium 65 (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium65.pdf). Colony colour was determined by comparing with ISCC–NBS colour charts (Kelly, 1964). Morphological characteristics were observed by light microscopy (ECLIPSE E200; Nikon) and electron microscopy (S-3400N; Hitachi) using cultures grown on ISP 3 agar at 28 °C for 14 days. Sporulation was assessed by light microscopic (ECLIPSE E200; Nikon) observation of cells suspended in phosphate buffer (pH 7.0, 1 mM). The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, utilization of sole carbon and nitrogen sources, degradation of catalase, esterase and urease were tested as described by Smibert & Krieg (1994). Growth at different temperatures (4, 16, 18, 22, 28, 32, 35 and 37 °C) was determined on DSMZ medium 65 after incubation for 14 days. Growth tests for pH range [4–12 in 1 pH unit intervals, using the buffer system described by Xie et al. (2012)] and NaCl tolerance [0–11 % (w/v) in 1 % intervals] were determined in ISP 2 at 28 °C for 14 days on a rotary shaker.

Biomass for chemical studies was prepared by growing the strain in ISP 2 medium in Erlenmeyer flasks for 7 days at 28 °C. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isolates of diamino-pimelic acid in the whole-cell hydrolysates were derivatized according to McKerrow et al. (2000) and analysed by HPLC using an Agilent TC-C18 Column (250 × 4.6 mm i.d. 5 μm) with a mobile phase consisting of acetonitrile/0.05 mol l⁻¹ phosphate buffer pH 7.2 (15:85, v/v) at a flow rate of 0.5 ml min⁻¹. The peak detection used an Agilent G1321A fluorescence detector with a 365 nm excitation wavelength and 455 nm longpass emission filters. Whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). Phospholipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaphenones were extracted from freeze-dried biomass and purified according to Collins (1985). Extracts were analysed by a HPLC-UV method using an Agilent Extend-C18 Column (250 × 4.6 mm i.d. 5 μm), typically at 270 nm. The mobile phase was acetonitrile/propyl alcohol (60:40, v/v) and the flow rate was set to 1.0 ml min⁻¹ and the run time was 60 min. The injection volume was 20 μl, and the chromatographic column was controlled at 40 °C (Wu et al., 1989). Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) after culture in ISP 2 at 28 °C for 14 days on a rotary shaker and were analysed by GC-MS using the method of Xiang et al. (2011).

Genomic DNA of strain NEAU-B-8T was extracted as described previously by Lee et al. (2003) and PCR amplification of the 16S rRNA gene was carried out using the method of Loqman et al. (2009). The PCR product was purified, cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). An almost full-length 16S rDNA gene sequence (1526 nt) was obtained. The sequence was aligned with reference strains using the CLUSTAL X 1.83 program. Phylogenetic trees were generated with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA software version 5.05 (Tamura et al., 2011a) with 1369 nt. The stability of the clades in the trees was appraised using a bootstrap analysis with 1000 repeats (Felsenstein, 1985). A distance matrix was generated using Kimura’s two-parameter model (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The EzTaxon-e server (Kim et al., 2012) was employed to identify the phylogenetic neighbours and calculate the pairwise 16S rRNA gene sequence similarities.

The G+C content of the genomic DNA was determined using the thermal denaturation (Tm) method (Mandel & Marmur, 1968) with Escherichia coli JM109 used as the control. DNA–DNA hybridization between isolate NEAU-B-8T and A. lutea JCM 17982T was carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 x 6 multicell changer and a temperature controller with in-situ temperature probe (Varian). The concentration and purity of DNA samples were determined by measuring the optical density at 260, 280 and 230 nm. The DNA samples used for hybridization were diluted to OD₂₆₆ around 1.0 using 0.1 x SSC (saline 117sodium citrate buffer), then were sheared using a JY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 cycles). The DNA renaturation rates were determined in 2 x SSC at 70 °C.

Morphological observation of a 14-day culture of strain NEAU-B-8T grown on DSMZ medium 65 revealed that aerial mycelium was abundant and substrate mycelium was well-developed without fragmentation. The aerial mycelium fragmented into rod-shaped elements and was pale to brilliant orange yellow in colour. Short spore chains were formed directly on the aerial mycelium. The strain displayed bud-like structures of the spore chains (0.32–0.64 x 0.9–1.3 μm) (Fig. S1, available in the online Supplementary Material). The spore surface was smooth. Sporangia were not found. Strain NEAU-B-8T showed good growth on ISP 3, Maltose Bennett’s agar and DSMZ medium 65, but poor
growth on ISP 2, ISP 4, ISP 6, ISP 7 and nutrient agar. Diffusible pigments or melanin were not formed on the tested media. Growth of strain NEAU-B-8\textsuperscript{T} occurred at pH 5–12 and in the presence of 0–10 % (w/v) NaCl, with optimum growth at pH 7.0 and 0 % NaCl. The temperature range for growth was 20–28 °C, with the optimum temperature being 28 °C. Detailed physiological characteristics are presented in the species description.

Strain NEAU-B-8\textsuperscript{T} contained meso-diaminopimelic acid in the cell wall. The whole-cell hydrolysate contained arabinose, glucose, galactose and xylose. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and phosphatidylinositol (phospholipid type II sensu). The predominant menaquinones were MK-8(H4) (98.2 %) and MK-8(H2) (1.8 %). The fatty acids were iso-C\textsubscript{16:0} (44.8 %), C\textsubscript{16:0} (21.2 %), C\textsubscript{17:0}ω7c (16.9 %), C\textsubscript{18:0} (9.7 %) and C\textsubscript{16:1}ω7c (7.4 %). Mycolic acids were not detected. The DNA G+C content was 69.8±0.3 mol%. All the morphological characteristics and chemotaxonomic data showed that strain NEAU-B-8\textsuperscript{T} should be assigned to the genus \textit{Actinomycetospora}.

Based on EzTaxon-e analysis, the 16S rRNA gene sequence (1526 nt) showed that strain NEAU-B-8\textsuperscript{T} belongs to the genus \textit{Actinomycetospora} and was most closely related to the \textit{Actinomycetospora rhizophila} TT07I-57\textsuperscript{T} (98.4 %), \textit{Actinomycetospora straminea} IY07-55\textsuperscript{T} (98.3 %) and \textit{Actinomycetospora chibensis} TT04-21\textsuperscript{T} (98.2 %). The phylogenetic tree based on 16S rRNA gene sequences (1369 nt) showed that strain NEAU-B-8\textsuperscript{T} belongs to the \textit{Actinomycetospora} genus. The phylogenetic tree showing the phylogenetic position of strain NEAU-B-8\textsuperscript{T} and related taxa based on 16S rRNA gene sequences (1369 nt). Asterisks indicate branches that were also recovered using the maximum-likelihood method. Bootstrap values ≥50 % (based on 1000 replications) are shown at branch points. \textit{Pseudonocardia thermophila} DSM 43832\textsuperscript{T} was used as the outgroup. Bar, 0.005 substitutions per nucleotide position.

In conclusion, it is evident from the genotypic and phenotypic data that strain NEAU-B-8\textsuperscript{T} represents a novel species of the genus \textit{Actinomycetospora}, for which the name \textit{Actinomycetospora rhizophila} sp. nov. is proposed.

**Description of \textit{Actinomycetospora rhizophila} sp. nov.**

\textit{Actinomycetospora rhizophila} (rhi.zo’ phi.la. Gr. n. rhiza a root; Gr. adj. philos loving; N.L. fem. adj. rhizophila root-loving).

Aerobic, Gram-stain-positive actinomycete that forms well-developed mycelium and aerial hyphae that differentiate into spore chains, formed by more than three spores (0.32–0.64 × 0.9–1.3 μm). Grows well on ISP 3, Maltose Bennett’s agar and DSMZ medium 65. Poor growth is observed on ISP 2, ISP 4, ISP 6, ISP 7 and nutrient agar. Diffusible pigments or melanin are not formed on all tested media. L-Arabinose, D-fructose, L-rhamnose, D-ribose and D-xylose are utilized as sole carbon sources as summarized in Table 1.
The type strain is NEAU-B-8T (=CGMCC 4.7134T =DSM 46673T), isolated from rhizosphere soil of a peace lily (Spathiphyllum Kochii) collected from Heilongjiang province, north-east China. The DNA G+C content of the type strain is 69.80±0.3 mol%.

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