Description of *Actinomycetospora chibensis* sp. nov., *Actinomycetospora chlora* sp. nov., *Actinomycetospora cinnamomea* sp. nov., *Actinomycetospora corticicola* sp. nov., *Actinomycetospora lutea* sp. nov., *Actinomycetospora straminea* sp. nov. and *Actinomycetospora succinea* sp. nov. and emended description of the genus *Actinomycetospora*

Tomohiko Tamura,1 Yuumi Ishida,1 Moriyuki Hamada,1 Misa Otoguro,1 Hideki Yamamura,2 Masayuki Hayakawa2 and Ken-ichiro Suzuki1

Correspondence
Tomohiko Tamura
tamura-tomohiko@nite.go.jp

1NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

2Division of Applied Biological Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Takeda-4, Kofu 400-8511, Japan

Eight actinomycete strains that form bud-like spore chains were isolated from various samples collected in Japan. Phylogenetically, the isolates formed a single clade with the type strain of *Actinomycetospora chiangmaiensis* according to 16S rRNA gene sequence analysis. The isolates contained meso-diaminopimelic acid, D-glutamic acid and D- and L-alanine in the cell-wall peptidoglycan, arabinoise and galactose as characteristic sugars, phosphatidylcholine and phosphatidylethanolamine as diagnostic phospholipids, MK-8(H4) as the predominant isoprenoid quinone, iso-C16:0 as the major cellular fatty acid and DNA G+C contents of 72–74 mol%. *Actinomycetospora chiangmaiensis*, the type species of the genus *Actinomycetospora*, was also found to contain MK-9(H4) predominantly in our study, although it was earlier reported to contain MK-9(H4) as the predominant isoprenoid quinone. On the basis of the morphological, physiological, chemotaxonomic, phylogenetic and DNA–DNA hybridization data, we concluded that the isolates can be accommodated in the genus *Actinomycetospora* with emendation of the description of the genus and are assigned to the following seven novel species: *Actinomycetospora chibensis* sp. nov. (type strain TT04-21^T^ = NBRC 103694^T^ = KACC 14256^T^), *Actinomycetospora chlora* sp. nov. (type strain TT07I-57^T^ = NBRC 105900^T^ = KACC 14252^T^), *Actinomycetospora cinnamomea* sp. nov. (type strain IT07I-57^T^ = NBRC 105527^T^ = KACC 14250^T^), *Actinomycetospora corticicola* sp. nov. (type strain 014-5^T^ = NBRC 103689^T^ = KACC 14253^T^), *Actinomycetospora lutea* sp. nov. (type strain TT00-04^T^ = NBRC 103690^T^ = KACC 14254^T^), *Actinomycetospora straminea* sp. nov. (type strain 014-5^T^ = NBRC 105528^T^ = KACC 14251^T^) and *Actinomycetospora succinea* sp. nov. (type strain TT00-49^T^ = NBRC 103691^T^ = KACC 14255^T^).

The genus *Actinomycetospora* was proposed by Jiang et al. (2008) to accommodate actinomycetes that form short spore chains on the substrate mycelium and contain meso-diaminopimelic acid (*meso*-A2pm), arabinoise and galactose in the cell wall (cell-wall chemotype IV), phosphatidylcholine as a diagnostic phospholipid and MK-9(H4) as a predominant menaquinone. At present, the only member...
of the genus is the type species *Actinomycetospora chiangmaiensis*, which was originally isolated from soil of a tropical rainforest in northern Thailand (Jiang et al., 2008).

During the course of an ecological study of actinomycetes in nature, eight actinomycete strains were isolated from soil and bark samples collected from subtropical islands and temperate areas of Japan (Table 1). All strains were isolated by the yeast extract-SDS method (Hayakawa & Nonomura, 1989) using humic acid-vitamin (HV) agar medium (Hayakawa & Nonomura, 1987). *A. chiangmaiensis* NBRC 104400T was used as a reference strain in our studies.

The 16S rRNA gene was amplified by PCR and sequenced as described previously (Tamura & Hatano, 2001). Phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). 16S rRNA gene sequences were aligned with published sequences retrieved from the DNA Data Bank of Japan (DDBJ) using CLUSTAL_X (Thompson et al., 1997), and sequences were edited manually using BioEdit version 7.0.9 (Hall, 1999). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms using the MEGA 4.1 program (Tamura et al., 2007). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replications.

16S rRNA gene sequence analyses revealed that the isolated strains formed a monophyletic cluster with *A. chiangmaiensis* YIM 0006T that had 100 % bootstrap support (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The binary similarity between strain 014-5T and *A. chiangmaiensis* YIM 0006T was 98.8 %, and similarities with other isolates ranged from 97.3 to 97.9 %. The similarity among our isolates, except strain 014-5T, ranged from 98.2 to 100 %. The 16S rRNA gene sequences of strains TT00-04T and TT01-72 were 100 % similar. All eight isolates and *A. chiangmaiensis* YIM 0006T showed 95.1 % similarity or less to *Pseudonocardia* strains.

For chemotaxonomic analyses, cells were grown in yeast extract-glucose broth (Hatano et al., 2003) on a rotary shaker at 28 °C for 5 days. Whole-cell sugar patterns, cell-wall amino acids, menaquinones, the acyl type of peptidoglycan, mycolic acids and DNA base compositions were analysed as described previously (Tamura et al., 1994). The isoprenoid quinone was determined by using liquid chromatography/mass spectrometry (LC/MS) according to Shimadzu Application Data Sheet no. 010 (Shimadzu, 2010). Cellular fatty acid methyl esters were prepared and analysed according to the protocol of the MIDI Sherlock Microbial Identification System (Sasser, 1990; MIDI, 2002). The eight isolates and *A. chiangmaiensis* NBRC 104400T contained MK-8(H4) as the predominant quinone. Arabinose, galactose and glucose were present as whole-cell sugars. The cell-wall peptidoglycan contained meso-A8pm, D- and L-alanine and D-glutamic acid. The major fatty acid present in these strains was iso-C16:0. The cellular fatty acid compositions of the isolates are shown in Supplementary Table S1. The phospholipids detected were phosphatidylethanolamine, phosphatidylcholine and diphasphatidylglycerol (phospholipid type III sensu Lechevalier et al., 1977). The DNA G+C content of the isolates ranged from 72.8 to 74.2 mol%.

Morphological characteristics were observed by scanning electron microscopy as described previously (Tamura et al., 1994). The isolates formed spore chains directly on the vegetative mycelium, which were observed to be bud-like, similar to those of *A. chiangmaiensis*. The spores were oval and rod-shaped with a smooth surface, 0.5–1.0 × 0.7–2.0 μm. Swollen spores (1.0–2.0 × 1.5–2.0 μm) were sometimes observed on the tips of the spore chains. Motile cells were not observed. Phylogenetic analysis, chemotaxonomic

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source, locality and year of isolation</th>
<th>16S rRNA gene accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinomycetospora chibensis</em> sp. nov. TT04-21T</td>
<td>Paddy soil; Mobara, Chiba, Japan; 2004</td>
<td>AB514517</td>
</tr>
<tr>
<td><em>Actinomycetospora chlora</em> sp. nov. TT071-57T</td>
<td>Paddy soil; Iriomote Island, Okinawa, Japan; 2007</td>
<td>AB514519</td>
</tr>
<tr>
<td><em>Actinomycetospora cinnamomea</em> sp. nov. IY07-53T</td>
<td>Paddy soil; Iriomote Island; 2007</td>
<td>AB514520</td>
</tr>
<tr>
<td><em>Actinomycetospora corticicola</em> sp. nov. 014-5T</td>
<td>Cortex of mangrove tree (<em>Kandelia candel</em>); Iriomote Island; 1996</td>
<td>AB514513</td>
</tr>
<tr>
<td><em>Actinomycetospora lutea</em> sp. nov. TT00-04T</td>
<td>Vegetable field soil; Amami Island, Kagoshima, Japan; 2000</td>
<td>AB514515</td>
</tr>
<tr>
<td>TT01-72</td>
<td>Pasture soil; Iriomote Island; 2001</td>
<td>AB514516</td>
</tr>
<tr>
<td><em>Actinomycetospora straminea</em> sp. nov. IY07-55T</td>
<td>Paddy soil; Iriomote Island; 2007</td>
<td>AB514518</td>
</tr>
<tr>
<td><em>Actinomycetospora succinea</em> sp. nov. TT00-49T</td>
<td>Vegetable field soil; Amami Island; 2000</td>
<td>AB514514</td>
</tr>
<tr>
<td><em>Actinomycetospora chiangmaiensis</em> NBRC 104400T</td>
<td>Rainforest soil; Thailand; NK</td>
<td>AM398646</td>
</tr>
</tbody>
</table>

NK, Not known.
Actinomycetospora chibensis TT04-21T (ABS14517)
Actinomycetospora straminea IY07-55T (ABS14518)
Actinomycetospora chlora TT07I-57T (ABS14519)
Actinomycetospora cinnamomea IY07-53T (ABS14520)
Actinomycetospora succinea TT00-49T (ABS14514)
Actinomycetospora lutea TT00-04T (ABS14515)
Actinomycetospora lutea TT01-72 (ABS14516)
Actinomycetospora chiangmaiensis IY07-53T (AM398646)
Actinomycetospora corticicola 014-5T (ABS14513)

Pseudonocardia thermophilia NRRL B-1978T

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences of Actinomycetospora strains. The tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). The sequence of Pseudonocardia thermophilia NRRL B-1978T was used as the outgroup. Numbers on branches indicate confidence limits (expressed as percentages) estimated by bootstrap analysis with 1000 replicates; only values >70% are shown. Filled circles indicate that the corresponding nodes (groupings) were also recovered in the maximum parsimony tree. An extended neighbour-joining tree is available as Supplementary Fig. S1. Bar, 0.01 K\textsubscript{muc}.

The microplate hybridization method developed by Ezaki et al. (1988, 1989) was applied to determine DNA–DNA relatedness. The relatedness among A. chiangmaiensis NBRC 104400\textsuperscript{T} and strains 014-5\textsuperscript{T}, IY07-53\textsuperscript{T}, TT07I-57\textsuperscript{T}, TT00-49\textsuperscript{T}, TT00-04\textsuperscript{T}, IY07-55\textsuperscript{T} and TT04-21\textsuperscript{T} ranged from 2 to 38% (Supplementary Table S3).

The isolates displayed morphological and chemotaxonomic characteristics similar to those of A. chiangmaiensis NBRC 104400\textsuperscript{T}. In the paper that originally described the genus Actinomycetospora, the predominant menaquinone was reported to be MK-9(H\textsubscript{4}) (Jiang et al., 2008). However, in our study, the predominant quinone of A. chiangmaiensis NBRC 104400\textsuperscript{T} was MK-8(H\textsubscript{4}) rather than MK-9(H\textsubscript{4}). The predominant quinone detected in all of our isolates was also MK-8(H\textsubscript{4}). Therefore, it is concluded that the predominant quinone of the genus Actinomycetospora is MK-8(H\textsubscript{4}). In addition, phosphatidylethanolamine as well as phosphatidylcholine was detected in all strains associated with Actinomycetospora.

DNA–DNA relatedness between strains TT00-04\textsuperscript{T} and TT01-72 was 63% (reciprocal value 83%). Strains TT00-04\textsuperscript{T} and TT01-72 were therefore identified as members of the same species. The chemotaxonomic characteristics as well as 16S rRNA gene sequence analyses show unambiguously that these isolates are affiliated with the genus Actinomycetospora. Phenotypic differences, the results of the DNA–DNA pairing studies and differences in 16S rRNA gene sequences indicate clearly that these isolates represent seven novel species belonging to the genus Actinomycetospora: Actinomycetospora chibensis sp. nov. (type strain TT04-21\textsuperscript{T}), Actinomycetospora chlora sp. nov. (type strain TT07I-57\textsuperscript{T}), Actinomycetospora cinnamomea sp. nov. (type strain IY07-53\textsuperscript{T}), Actinomycetospora corticicola sp. nov. (type strain 014-5\textsuperscript{T}), Actinomycetospora lutea sp. nov. (type strain TT00-04\textsuperscript{T}) and Actinomycetospora succinea sp. nov.

Table 2. Differential characteristics of the eight novel isolates and A. chiangmaiensis NBRC 104400\textsuperscript{T}

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pyrrolidonyl arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>W</td>
<td>-</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Urea hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Esterase (C-4)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Valine aminopeptidase</td>
<td>W</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>W</td>
<td>W</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythritol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Turanose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>l-Arabinol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth pH</td>
<td>6–7</td>
<td>5–8</td>
<td>5–8</td>
<td>5–9</td>
<td>5–8</td>
<td>5–8</td>
<td>6–8</td>
<td>5–9</td>
<td>ND</td>
</tr>
<tr>
<td>Tolerance of NaCl (%) (w/v)</td>
<td>0–1</td>
<td>0–1</td>
<td>0–7</td>
<td>0–7</td>
<td>0–1</td>
<td>0–1</td>
<td>0–5</td>
<td>0–7</td>
<td>ND</td>
</tr>
</tbody>
</table>

The isolates: 1, A. chibensis sp. nov. TT04-21\textsuperscript{T}; 2, A. chlora sp. nov. TT07I-57\textsuperscript{T}; 3, A. cinnamomea sp. nov. IY07-53\textsuperscript{T}; 4, A. corticicola sp. nov. 014-5\textsuperscript{T}; 5, A. lutea sp. nov. TT00-04\textsuperscript{T}; 6, A. lutea sp. nov. TT01-72; 7, A. straminea sp. nov. IY07-55\textsuperscript{T}; 8, A. succinea sp. nov. TT00-49\textsuperscript{T}; 9, A. chiangmaiensis NBRC 104400\textsuperscript{T}. +, Positive; −, negative; w, weakly positive; V, variable; ND, not determined. Data were obtained in this study.
sp. nov. (type strain TT00-04T; reference strain TT01-72), *Actinomycetospora straminea* sp. nov. (type strain IY07-55T) and *Actinomycetospora succinea* sp. nov. (type strain TT00-49T).

**Emended description of the genus Actinomycetospora Jiang et al. 2008**

The description of the genus is as given by Jiang et al. (2008), with the following modifications. The predominant menaquinone is MK-8(H4). Phosphatidylcholine and phosphatidylethanolamine are the diagnostic phospholipids.

**Description of Actinomycetospora chibensis sp. nov.**

*Actinomycetospora chibensis* (chi.ben’sis. N.L. fem. adj. chibensis of or belonging to Chiba, a Japanese prefecture, from where the type strain originated).

The surface of colonies is powdery. The colour of the substrate mycelium is vivid to light yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 15–28 °C, pH 6.0–7.0 and NaCl concentrations up to 1%. D-Arabinitol, aesculin ferric citrate, glycerol, 2-ketoglucuronate, 5-ketoglucuronate, D-lyxose, D-mannitol, L-rhamnose, D-ribose, D-sorbitol, starch and turanose are utilized as sole carbon sources. Positive for nitrate reduction, gelatin hydrolysis, alkaline phosphatase, esterase lipase (C-8), a-glucoamylase, leucine aminopeptidase, pyrazinamidase, pyrrolidonyl arylamidase and valine aminopeptidase, but negative for aesculin hydrolysis and urea hydrolysis. The major cellular fatty acids are iso-C16:0 and C16:1 cis-9. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT071-57T (＝NBRC 105900T = KACC 14252T), which was isolated from a paddy soil on Iriomote Island, Okinawa, Japan.

**Description of Actinomycetospora cinnamomea sp. nov.**

*Actinomycetospora cinnamomea* (cin.na.mo’me.a. L. n. cinnamonum cinnamon; L. fem. suff. -a suffix used with various meanings; N.L. fem. adj. cinnamomea cinnamon-coloured).

The surface of the colonies is powdery. The colour of the substrate mycelium is orange. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 15–37 °C, pH 5.0–8.0 and NaCl concentrations up to 7%. D-Arabinitol, D-fructose, glycerol, D-mannitol, D-mannitol and D-sorbitol are utilized as sole carbon sources. Positive for gelatin hydrolysis, acid phosphatase, alkaline phosphatase, esterase (C-4), esterase lipase (C-8), a-glucosidase, leucine aminopeptidase and pyrazinamidase, but negative for ascorbic acid hydrolysis and urea hydrolysis. The major cellular fatty acids are iso-C16:0 and C17:1 cis-9. The DNA G+C content of the type strain is 74 mol%.

The type strain is IY07-53T (＝NBRC 105527T = KACC 142501T), which was isolated from paddy soil on Iriomote Island, Okinawa, Japan.

**Description of Actinomycetospora corticicola sp. nov.**

*Actinomycetospora corticicola* [cor.ti.ci’co.la. L. n. cortex -icus the bark; L. suff. -cola (from L. n. incola) inhabitant, dweller; N.L. n. corticicola inhabitant of bark].

The surface of the colonies is powdery. The colour of the substrate mycelium is orange–yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 10–37 °C, pH 5.0–9.0 and NaCl concentrations up to 7%. D-Arabinitol, D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, melezitose, D-sorbitol, sucrose, trehalose, turanose and xylitol are utilized as sole carbon sources. Positive for ascorbic acid hydrolysis, gelatin hydrolysis, acid phosphatase, alkaline phosphatase, esterase (C-8), a-glucosidase, leucine aminopeptidase and pyrazinamidase, but negative for nitrate reduction. The major cellular fatty acids are iso-C16:0 and 10-methyl C16:0. The DNA G+C content of the type strain is 74 mol%.

The type strain is 014-5 T (＝NBRC 103694T = KACC 142561T), which was isolated from a paddy field soil in Mabara, Chiba, Japan.
Description of *Actinomycetospora lutea* sp. nov.

*Actinomycetospora lutea* (lu’t’e.a. L. fem. adj. *lutea* yellow-coloured).

The surface of the colonies is powdery. The colour of the substrate mycelium is vivid to light yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 20–37 °C and pH 5.0–8.0. D-Arabinol, D-arabitol, erythritol, D-fructose, D-galactose, D-glucose, D-mannose, D-sorbitol, and turanose are utilized as sole carbon sources. Positive for gelatin hydrolysis, urea hydrolysis, esterase lipase (C-8), x-glucosidase, leucine aminopeptidase, phosphohydrolase and pyrazinamidase, but negative for ascinulin hydrolysis, nitrate reduction and urea hydrolysis. The major cellular fatty acids are iso-C_{16:0}, C_{17:1} cis-9, C_{15:0}, and C_{16:0} cis-9. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT00-04^T (=NBRC 103690^T =KACC 14254^T), which was isolated from vegetable field soil on Amami Island, Kagoshima, Japan. Strain TT01-72 (=NBRC 103692), from a similar source, is a second strain of the species.

Description of *Actinomycetospora straminea* sp. nov.

*Actinomycetospora straminea* (stra.mi’ne.a. L. fem. adj. *straminea* made of straw, intended to mean straw-coloured).

The surface of the colonies is powdery. The colour of the substrate mycelium is pale greenish yellow to yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 15–37 °C, pH 5.0–9.0 and at NaCl concentrations up to 7 %. D-Adonitol, D-arabitol, erythritol, D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, D-sorbitol, sucrose, trehalose and xylitol are utilized as sole carbon sources. Positive for gelatin hydrolysis, urea hydrolysis, esterase lipase (C-8), x-glucosidase, leucine aminopeptidase, pyrazinamidase and valine aminopeptidase, but negative for ascinulin hydrolysis and nitrate reduction. The major cellular fatty acids are iso-C_{16:0} and C_{17:1} cis-9. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT00-49^T (=NBRC 103691^T =KACC 14255^T), which was isolated from vegetable field soil on Amami Island, Kagoshima, Japan.

Acknowledgements

This study was supported in part by the Japan Society for the Promotion of Science [Grant-in-Aid for Scientific Research (C) (2) no. 11660326] and a research grant from the Institute for Fermentation, Osaka (IFO).

References


