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

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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 *Actinomycesetospora 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, arabinose and galactose as characteristic sugars, phosphatidylcholine and phosphatidylethanolamine as diagnostic phospholipids, MK-8(H₄) as the predominant isoprenoid quinone, iso-C_{16:0} as the major cellular fatty acid and DNA G + C contents of 72–74 mol%. *Actinomycesetospora chiangmaiensis*, the type species of the genus *Actinomycesetospora*, was also found to contain MK-8(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 *Actinomycesetospora* with emendation of the description of the genus and are assigned to the following seven novel species: *Actinomycesetospora chibensis* sp. nov. (type strain TT04-21^T = NBRC 103694^T = KACC 14256^T), *Actinomycesetospora chlora* sp. nov. (type strain TT07I-57^T = NBRC 105900^T = KACC 14252^T), *Actinomycesetospora cinnamomea* sp. nov. (type strain IY07-53^T = NBRC 105527^T = KACC 14250^T), *Actinomycesetospora corticicola* sp. nov. (type strain 014-5^T = NBRC 103689^T = KACC 14253^T), *Actinomycesetospora lutea* sp. nov. (type strain TT00-04^T = NBRC 103690^T = KACC 14254^T), *Actinomycesetospora straminea* sp. nov. (type strain IY07-55^T = NBRC 105528^T = KACC 14251^T) and *Actinomycesetospora succinea* sp. nov. (type strain TT00-49^T = NBRC 103691^T = KACC 14255^T).

Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the novel strains reported in this study are AB514513–AB514520, as detailed in Table 1.

A supplementary figure and three supplementary tables are available with the online version of this paper.

The genus *Actinomycesetospora* 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), arabinose 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 *Actinomycesporia 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 104400^T 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 0006^T that had 100 % bootstrap support (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The binary similarity between strain 014-5^T and *A. chiangmaiensis* YIM 0006^T was 98.8 %, and similarities with other isolates ranged from 97.3 to 97.9 %. The similarity among our isolates, except strain 014-5^T, ranged from 98.2 to 100 %. The 16S rRNA gene sequences of

strains TT00-04^T and TT01-72 were 100 % similar. All eight isolates and *A. chiangmaiensis* YIM 0006^T 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 104400^T contained MK-8(H₄) as the predominant quinone. Arabinose, galactose and glucose were present as whole-cell sugars. The cell-wall peptidoglycan contained *meso*-A₂pm, D- and L-alanine and D-glutamic acid. The major fatty acid present in these strains was iso-C_{16:0}. The cellular fatty acid compositions of the isolates are shown in Supplementary Table S1. The phospholipids detected were phosphatidylethanolamine, phosphatidylcholine and diphosphatidylglycerol (phospholipid type PIII *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 1. Actinomycete strains used in this study

Strain	Source, locality and year of isolation	16S rRNA gene accession no.
<i>Actinomycesporia chibensis</i> sp. nov. TT04-21 ^T	Paddy soil; Mobara, Chiba, Japan; 2004	AB514517
<i>Actinomycesporia chlora</i> sp. nov. TT07I-57 ^T	Paddy soil; Iriomote Island, Okinawa, Japan; 2007	AB514519
<i>Actinomycesporia cinnamomea</i> sp. nov. IY07-53 ^T	Paddy soil; Iriomote Island; 2007	AB514520
<i>Actinomycesporia corticicola</i> sp. nov. 014-5 ^T	Cortex of mangrove tree (<i>Kandelia candel</i>); Iriomote Island; 1996	AB514513
<i>Actinomycesporia lutea</i> sp. nov. TT00-04 ^T	Vegetable field soil; Amami Island, Kagoshima, Japan; 2000	AB514515
TT01-72	Pasture soil; Iriomote Island; 2001	AB514516
<i>Actinomycesporia straminea</i> sp. nov. IY07-55 ^T	Paddy soil; Iriomote Island; 2007	AB514518
<i>Actinomycesporia succinea</i> sp. nov. TT00-49 ^T	Vegetable field soil; Amami Island; 2000	AB514514
<i>Actinomycesporia chiangmaiensis</i> NBRC 104400 ^T	Rainforest soil; Thailand; NK	AM398646

NK, Not known.

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 thermophila* NRRL B-1978^T 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_{nuc} .

characteristics and morphological features indicated that the isolates belong to the genus *Actinomycetospora*.

Cultural and physiological characteristics were determined as described previously (Gordon *et al.*, 1974; Seino *et al.*, 1985) and by using API ZYM, API 50CH and API Coryne test strips (bioMérieux). Detailed results of these analyses are provided in Table 2, Supplementary Table S2 and the species descriptions. The mass colour of strain IY07-53^T was orange, that of TT07I-57^T and IY07-55^T was light yellow to greenish yellow and the other isolates were yellow. Growth and physiological characteristics differed between all strains except TT00-04^T and TT01-72, which showed similar characteristics.

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^T and strains 014-5^T, IY07-53^T, TT07I-57^T, TT00-49^T, TT00-04^T, IY07-55^T and TT04-21^T ranged from 2 to 38 % (Supplementary Table S3).

The isolates displayed morphological and chemotaxonomic characteristics similar to those of *A. chiangmaiensis* NBRC 104400^T. In the paper that originally described the genus *Actinomycetospora*, the predominant menaquinone was reported to be MK-9(H₄) (Jiang *et al.*, 2008). However, in our study, the predominant quinone of *A. chiangmaiensis* NBRC 104400^T was MK-8(H₄) rather than MK-9(H₄). The predominant quinone detected in all of our isolates was also MK-8(H₄). Therefore, it is concluded that the predominant quinone of the genus *Actinomycetospora* is MK-8(H₄). In addition, phosphatidylethanolamine as well as phosphatidylcholine was detected in all strains associated with *Actinomycetospora*.

DNA–DNA relatedness between strains TT00-04^T and TT01-72 was 63 % (reciprocal value 83 %). Strains TT00-04^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^T), *Actinomycetospora chlora* sp. nov. (type strain TT07I-57^T), *Actinomycetospora cinnamomea* sp. nov. (type strain IY07-53^T), *Actinomycetospora corticicola* sp. nov. (type strain 014-5^T), *Actinomycetospora lutea*

Table 2. Differential characteristics of the eight novel isolates and *A. chiangmaiensis* NBRC 104400^T

Strains: 1, *A. chibensis* sp. nov. TT04-21^T; 2, *A. chlora* sp. nov. TT07I-57^T; 3, *A. cinnamomea* sp. nov. IY07-53^T; 4, *A. corticicola* sp. nov. 014-5^T; 5, *A. lutea* sp. nov. TT00-04^T; 6, *A. lutea* sp. nov. TT01-72; 7, *A. straminea* sp. nov. IY07-55^T; 8, *A. succinea* sp. nov. TT00-49^T; 9, *A. chiangmaiensis* NBRC 104400^T. +, Positive; –, negative; w, weakly positive; v, variable; ND, not determined. Data were obtained in this study.

Characteristic	1	2	3	4	5	6	7	8	9
Nitrate reduction	+	–	+	–	–	–	+	–	–
Pyrrolidonyl arylamidase	+	+	–	+	–	–	+	w	–
Aesculin hydrolysis	–	–	–	+	–	–	–	–	+
Urea hydrolysis	–	–	–	+	–	–	–	+	+
Esterase (C-4)	+	–	+	–	+	+	+	w	+
Valine aminopeptidase	w	+	–	+	–	–	+	+	w
Utilization of:									
Erythritol	–	–	–	–	v	v	–	+	–
Trehalose	–	–	–	+	v	v	+	+	+
Turanose	+	+	–	+	+	+	+	v	+
L-Arabitol	–	–	–	v	–	v	+	–	–
Growth pH	6–7	5–8	5–8	5–9	5–8	5–8	6–8	5–9	ND
Tolerance of NaCl (% w/v)	0–1	0–1	0–7	0–7	0–1	0–1	0–5	0–7	ND
Growth temperature (°C)	15–28	15–28	15–37	10–37	20–28	20–37	10–28	15–37	ND

sp. nov. (type strain TT00-04^T; reference strain TT01-72), *Actinomycespora straminea* sp. nov. (type strain IY07-55^T) and *Actinomycespora succinea* sp. nov. (type strain TT00-49^T).

Emended description of the genus *Actinomycespora* 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 *Actinomycespora chibensis* sp. nov.

Actinomycespora 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-Arabitol, aesculin ferric citrate, glycerol, 2-ketogluconate, 5-ketogluconate, 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 (C-4), esterase lipase (C-8), α -glucosidase, leucine aminopeptidase, pyrazinamidase, pyrrolidonyl arylamidase and valine aminopeptidase, but negative for aesculin hydrolysis and urea hydrolysis. The major cellular fatty acids are iso-C_{16:0} and iso-C_{15:0}. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT04-21^T (=NBRC 103694^T =KACC 14256^T), which was isolated from a paddy field soil in Mobara, Chiba, Japan.

Description of *Actinomycespora chlora* sp. nov.

Actinomycespora chlora [chlo'ra. N.L. fem. adj. *chlora* (from Gr. adj. *khloros* -a -on) greenish-yellow, referring to the greenish-yellow to pale green colour on yeast extract-starch medium].

The surface of the colonies is powdery. The colour of the substrate mycelium is pale greenish yellow to vivid 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 5.0–8.0 and NaCl concentrations up to 1 %. L-Arabinose, D-arabitol, D-fructose, glycerol, D-ribose, sucrose and turanose are utilized as sole carbon sources. Positive for gelatin hydrolysis, acid phosphatase, esterase lipase (C-8), α -glucosidase, leucine aminopeptidase, pyrazinamidase, pyrrolidonyl arylamidase and valine aminopeptidase, but negative for aesculin hydrolysis, urea hydrolysis and nitrate reduction. The major cellular fatty

acids are iso-C_{16:0} and C_{16:1} *cis*-9. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT07I-57^T (=NBRC 105900^T =KACC 14252^T), which was isolated from a paddy soil on Iriomote Island, Okinawa, Japan.

Description of *Actinomycespora cinnamomea* sp. nov.

Actinomycespora cinnamomea (cin.na.mo'me.a. L. n. *cinnamomum* 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-Arabitol, D-fructose, glycerol, D-mannose, 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), α -glucosidase, leucine aminopeptidase and pyrazinamidase, but negative for aesculin hydrolysis and urea hydrolysis. 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 74 mol%.

The type strain is IY07-53^T (=NBRC 105527^T =KACC 14250^T), which was isolated from paddy soil on Iriomote Island, Okinawa, Japan.

Description of *Actinomycespora corticicola* sp. nov.

Actinomycespora corticicola [cor.ti.ci'co.la. L. n. *cortex* -icis 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-Arabitol, 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 aesculin hydrolysis, gelatin hydrolysis, urea hydrolysis, acid phosphatase, alkaline phosphatase, esterase lipase (C-8), α -glucosidase, leucine aminopeptidase, pyrazinamidase, pyrrolidonyl arylamidase and valine aminopeptidase, but negative for nitrate reduction. The major cellular fatty acids are iso-C_{16:0} and 10-methyl C_{16:0}. The DNA G+C content of the type strain is 74 mol%.

The type strain is 014-5^T (=NBRC 103689^T =KACC 14253^T), which was isolated from bark of a mangrove tree, *Kandelia candel*, on Iriomote Island, Okinawa, Japan.

Description of *Actinomycetospora lutea* sp. nov.

Actinomycetospora lutea (lu'te.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-Arabitol, D-fructose, D-glucose, D-mannitol, D-sorbitol and turanose are utilized as sole carbon sources. Positive for gelatin hydrolysis, acid phosphatase, esterase (C-4), esterase lipase (C-8), α -glucosidase, leucine aminopeptidase, phosphohydrolase and pyrazinamidase, but negative for aesculin 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_{16:1} *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 spore chains form loops or spirals. The substrate mycelium is long, well-developed and branched. Grows at 10–28 °C, pH 6.0–8.0 and NaCl concentrations up to 5 %. D-Arabitol, L-arabitol, L-fucose, gluconate, 5-ketogluconate, D-glucose, D-lyxose, D-mannitol, sucrose, trehalose and turanose are utilized as sole carbon sources. Positive for gelatin hydrolysis, nitrate reduction, acid phosphatase, cystine aminopeptidase, esterase (C-4), esterase lipase (C-8), α -glucosidase, leucine aminopeptidase, pyrazinamidase, pyrrolidonyl arylamidase and valine aminopeptidase, but negative for aesculin and urea hydrolysis. The major cellular fatty acids are iso-C_{16:0} and iso-C_{16:1}. The DNA G + C content of the type strain is 74 mol%.

The type strain is IY07-55^T (=NBRC 105528^T =KACC 14251^T), which was isolated from paddy field soil on Iriomote Island, Okinawa, Japan.

Description of *Actinomycetospora succinea* sp. nov.

Actinomycetospora succinea (suc.ci'ne.a. L. fem. adj. *succinea* of amber, intended to mean amber-coloured).

The surface of the colonies is powdery. The colour of the substrate mycelium is light to strong 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), α -glucosidase, leucine aminopeptidase, pyrazinamidase and valine aminopeptidase, but negative for aesculin 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.

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