Asanoa iriomotensis sp. nov., isolated from mangrove soil

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During a study of the distribution of actinomycetes in a mangrove zone, a strain forming spore chains borne on the tip of short sporophores arising directly from the agar surface was isolated from soil. The isolate contained glutamic acid, glycine, alanine and *meso*-diaminopimelic acid as cell-wall amino acids, menaquinone MK-10(H₆, H₈), fatty acid type 2d and xylose in the whole-cell hydrolysate. The 16S rRNA gene sequence of the isolate formed a monophyletic cluster with the members of the genus *Asanoa* in the family *Micromonosporaceae*. On the basis of morphological and chemotaxonomic characteristics, phylogenetic analysis and DNA–DNA hybridization, a novel species of the genus *Asanoa* is proposed for strain TT 97-02^T (=NBRC 100142^T=DSM 44745^T), *Asanoa iriomotensis* sp. nov.

The genus *Asanoa* was proposed by Lee & Hah (2002) to accommodate actinomycetes with *meso*-diaminopimelic acid (A₂pm) and 3-OH A₂pm in the cell wall, MK-10(H₆, H₈) as the major menaquinone and fatty acid type 2d and which belong to the family *Micromonosporaceae* by their 16S rRNA gene sequences. The genus was established with *Asanoa ferruginea* (basonym *Catellatospora ferruginea* Asano and Kawamoto 1986) and *Asanoa ishikariensis* (formerly '*Catellatospora ishikariense*') and was distinguished from the genus *Catellatospora* by members of the latter genus displaying major menaquinone MK-10(H₄) or MK-9(H₄, H₆) and fatty acid type 3b (Lee & Hah, 2002).

Mangroves are salt-adapted plants found along many of the world's tropical and subtropical coastlines (Dodd, 2000). In Japan, mangroves develop along estuaries in the subtropical zone, where sea water and river water mix. During a study of the diversity of actinomycetes that inhabit the mangrove zone, strain TT 97-02^T was isolated from soil around the roots of the mangrove *Bruguiera gymnorhiza*. Strain TT 97-02^T formed spore chains borne on the tip of short sporophores arising directly from the agar surface. The isolate formed a monophyletic cluster with the members of the genus *Asanoa* and had fatty acid type 2d and major menaquinone MK-10(H₆, H₈). This isolate represents a novel species of *Asanoa*, for which we propose the name *Asanoa iriomotensis*.

Strain TT 97-02^T was isolated on humic acid-vitamin (HV)

agar (Hayakawa & Nonomura, 1987) by the yeast extract/SDS method (Hayakawa & Nonomura, 1989) from soil around the roots of *B. gymnorhiza* growing along the Shiira River in Iriomote Island, Okinawa Prefecture, Japan. Freeze-dried cells for chemotaxonomic analyses were grown in yeast extract/glucose broth (10 g yeast extract and 10 g D-glucose per litre distilled water, pH 7.0) on a rotary shaker at 28 °C. *A. ferruginea* NBRC 14496^T and *A. ishikariensis* NBRC 14551^T were used as reference strains.

Cultural and physiological characteristics were determined as described previously (Gordon *et al.*, 1974; Seino *et al.*, 1985; Shirling & Gottlieb, 1966; Yokota *et al.*, 1993). Morphology was observed by scanning electron microscopy as described previously (Tamura *et al.*, 2000). Analyses of whole-cell sugar patterns, cell-wall amino acids, menaquinones, cellular fatty acids, isomers of A₂pm, acyl type of peptidoglycan, mycolic acid and DNA base composition were performed as described previously (Tamura *et al.*, 1994). The microplate hybridization method developed by Ezaki *et al.* (1988, 1989) was applied with minor modifications to determine DNA relatedness (Tamura *et al.*, 1999). PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Tamura & Hatano, 2001) with a model ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer's protocol. Phylogenetic analysis of 16S rRNA gene sequences was performed as described previously (Tamura & Hatano, 2001).

The isolate was a Gram-positive, non-acid-fast and aerobic organism with branched hyphae. A non-fragmenting substrate mycelium was formed. A 14-day-old culture grown on HV agar showed spore chains borne on the tip of short sporophores arising directly from the agar surface (see

Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Asanoa iriomotensis* TT 97-02^T is AB112081.

A scanning electron micrograph showing sporulation of strain TT 97-02^T is available as supplementary material in IJSEM Online.

supplementary figure in IJSEM Online). Motile spores were not observed.

The isolate exhibited good growth on yeast extract-malt extract agar (ISP medium 2), moderate growth on oatmeal agar (ISP medium 3), poor growth on inorganic salts-starch agar (ISP medium 4), glycerol-asparagine agar (ISP medium 5) and peptone-yeast extract-iron agar (ISP medium 6) and almost no growth on tyrosine agar (ISP medium 7). The isolate formed spore chains on water agar and HV agar, but not on yeast extract-malt extract agar, oatmeal agar, inorganic salts-starch agar, glycerol-asparagine agar, peptone-yeast extract iron agar or tyrosine agar.

Strain TT 97-02^T hydrolysed starch, but did not reduce nitrate. It grew at 20 and 30 °C, but not at 15 or 37 °C. The isolate utilized (–)-D-mannitol, (+)-D-melibiose, (+)-D-maltose, (+)-L-rhamnose, methyl α -D-glucoside, (+)-D-raffinose, (+)-D-galactose, (+)-D-mannose and glucose and grew weakly on (+)-L-arabinose and (+)-D-xylose, but did not utilize L-erythritol, adonitol, (+)-D-lactose, L-inositol, D-sorbitol or dulcitol as sole carbon sources. The isolate did not grow on 4 % NaCl. Hydrolysis of aesculin, decomposition of urea, utilization of sodium succinate, sodium oxalate, sodium malate and sodium citrate and growth on MacConkey agar were negative.

The cell walls contained *meso*-A₂pm, D-glutamic acid, glycine and L-alanine, indicating that the peptidoglycan is type A1 γ according to Schleifer & Kandler (1972). The isolate contained xylose, mannose, galactose and glucose as whole-cell sugars, but arabinose was not detected. The major menaquinones were MK-10(H₆) and MK-10(H₈). The major cellular fatty acids were anteiso-C_{15:0} (22.3 %), anteiso-C_{17:0} (19.6 %), iso-C_{15:0} (18.0 %), C_{17:0} (13.6 %) and iso-C_{16:0} (12.2 %), and small amounts of iso-C_{17:0}, C_{16:0}, C_{18:0}, C_{15:0} and iso-C_{14:0} were also detected. Mycolic acids were absent. Phosphatidylethanolamine was detected, but phospholipids including unidentified glucosamine and phosphatidylcholine were not detected, corresponding to phospholipid type II of Lechevalier *et al.* (1981). The acyl type of cell-wall polysaccharides was acetyl. The G+C content of the genomic DNA was 69 mol%.

The 16S rRNA gene sequence was determined for strain TT 97-02^T (positions 28–1525 according to the *Escherichia coli* numbering system; Brosius *et al.*, 1978). Positions at which secondary structures varied between strains and positions at which the sequence was not determined in some reference organisms were excluded from the analysis. The phylogenetic tree obtained by applying the neighbour-joining method to K_{nuc} values is depicted in Fig. 1. 16S rRNA gene sequence analysis revealed that the isolate fell in the cluster of the family *Micromonosporaceae* and formed a monophyletic cluster with members of the genus *Asanoa*. The isolate was closely related to *A. ferruginea* and *A. ishikariensis* (96.9–97.1 % similarity). In a DNA–DNA hybridization study, strain TT 97-02^T exhibited levels of DNA–DNA relatedness of 15–28 % with *A. ferruginea* NBRC 14496^T and *A. ishikariensis* NBRC 14551^T.

The previously known members of the genus *Asanoa* show poor sporulation that occurs only on tap water agar and glycerol/calcium malate agar (Asano & Kawamoto, 1986; Lee & Hah, 2002). Sporulation of strain TT 97-02^T was not observed on richly nutritious media but developed well on water agar and HV agar. Chemotaxonomic characteristics and phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate is a member of the genus *Asanoa*. The isolate could be distinguished from the two known species, *A. ferruginea* and *A. ishikariensis*, by its low relatedness in DNA–DNA hybridization, its cellular fatty acid profile and the utilization of some carbon sources (Table 1). On the basis of the data presented above, we propose that strain TT 97-02^T be classified in a novel species, *Asanoa iriomotensis* sp. nov.

Description of *Asanoa iriomotensis* sp. nov.

Asanoa iriomotensis (i.ri.o.mo.ten'sis. N.L. fem. adj. *iriomotensis* of Iriomote Island, Okinawa, Japan, the origin of the soil sample from which the type strain was isolated).

Spore chains borne on the tip of short sporophores arising directly from the agar surface develop on water agar and HV agar. Hydrolyses starch. Does not reduce nitrate. Grows at 20 and 30 °C, but not at 15 or 37 °C. Utilizes

Fig. 1. Phylogenetic tree, based on the neighbour-joining method (Saitou & Nei, 1987), derived from 16S rRNA gene sequences for members of the family *Micromonosporaceae*. *Streptomyces ambofaciens* ATCC 23877^T was used as the root organism. Scale bar, 0.01 K_{nuc} in nucleotide sequences. Numbers on branches are confidence limits (expressed as percentages) estimated from a bootstrap analysis with 1000 replicates.

Table 1. Diagnostic characteristics that differentiate *Asanoa iriomotensis* sp. nov. TT 97-02^T from other members of the genus *Asanoa*

Data for reference species were taken from Lee & Hah (2002). w, Weak growth.

Characteristic	Strain TT 97-02 ^T	<i>A. ferruginea</i>	<i>A. ishihikariensis</i>
Utilization of carbohydrates:			
Adonitol	—	+	+
(+)-D-Lactose	—	+	+
(+)-L-Arabinose	w	+	+
Dulcitol	—	—	+
Growth at 37 °C	—	+	—
Major cellular fatty acids	anteiso-C _{15:0} , anteiso-C _{17:0} , iso-C _{15:0} , C _{17:0} , iso-C _{16:0}	anteiso-C _{15:0} , C _{17:0} , iso-C _{16:0} , C _{17:1} , iso-C _{15:0}	anteiso-C _{15:0} , C _{17:1} , iso-C _{15:0} , C _{17:0}

(–)-D-mannitol, (+)-D-melibiose, (+)-D-maltose, (+)-L-rhamnose, methyl α-D-glucoside, (+)-D-raffinose, (+)-D-galactose, (+)-D-mannose and glucose. Does not grow on L-erythritol, adonitol, (+)-D-lactose, L-inositol, D-sorbitol or dulcitol. Phosphatidylethanolamine is present as the diagnostic phospholipid. Unsaturated fatty acids and 10-methylated fatty acids are not detected. The fatty acid profile is characterized by significant amounts of anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0}, C_{17:0} and iso-C_{16:0}. The G+C content of genomic DNA of the type strain is 69 mol%. Habitat is soil.

The type strain is TT 97-02^T (=NBRC 100142^T=DSM 44745^T).

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