Asanoa iriomotensis sp. nov., isolated from mangrove soil

Tomohiko Tamura and Takeshi Sakane

Biological Resource Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0812, Japan

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 97-02T (=NBRC 100142T = DSM 44745T), 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 ishikariensis’) and was distinguished from the genus Catellatospora by members of the latter genus displaying major menaquinone MK-10(H₆) or MK-9(H₆, H₈) 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 97-02T was isolated from soil around the roots of the mangrove Bruguiera gymnorrhiza. Strain TT 97-02T 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 97-02T 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. gymnorrhiza growing along the Shira 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 14496T and A. ishikariensis NBRC 14551T 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, mycopic 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...
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\textsuperscript{T} hydrolysed starch, but did not reduce peptone-yeast extract iron agar or tyrosine agar. 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\textsuperscript{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 x-D-glucoside, (−)-D-raffinose, (−)-D-galactose, (+)-D-mannose and glucose and grew weakly on (−)-L-arabinose and (−)-D-xylene, but did not utilize L-erythritol, adonitol, (+)-D-lactose, l-mannitol, d-sorbitol or dulcitol as sole carbon sources. The isolate did not grow on 4 % NaCl. Hydrolysis of asascin, 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 \textit{meso}-A\textsubscript{3}pm, D-glutamic acid, glycine and l-alanine, indicating that the peptidoglycan is type A\textsubscript{1}\textgamma 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(H\textsubscript{6}) and MK-10(H\textsubscript{8}). The major cellular fatty acids were anteiso-C\textsubscript{15:0} (22.3 %), anteiso-C\textsubscript{17:0} (19.6 %), iso-C\textsubscript{15:0} (18.0 %), C\textsubscript{17:0} (13.6 %) and iso-C\textsubscript{16:0} (12.2 %), and small amounts of iso-C\textsubscript{17:0}, C\textsubscript{16:0}, C\textsubscript{18:0}, C\textsubscript{15:0} and iso-C\textsubscript{14:0} were also detected. Mycolic acids were absent. Phosphatidylethanolamine was detected, but phospholipids including unidentified glucosamine and phosphatidylcholine were not detected, corresponding to the peptidoglycan type II of \textit{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\textsuperscript{T} (positions 28–1525 according to the \textit{Escherichia coli} numbering system; Brosius \textit{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 \textit{K}\textsubscript{nuc} values is depicted in Fig. 1. The 16S rRNA gene sequence analysis revealed that the isolate fell in the cluster of the family \textit{Micromonosporaceae} and formed a monophyletic cluster with members of the genus \textit{Asanoa}. The isolate was closely related to \textit{A. ferruginea} and \textit{A. ishikariensis} (96–97\% similarity). In a DNA–DNA hybridization study, strain TT 97-02\textsuperscript{T} exhibited levels of DNA–DNA relatedness of 15–28 % with \textit{A. ferruginea} NBRC 14496\textsuperscript{T} and \textit{A. ishikariensis} NBRC 14551\textsuperscript{T}.

The previously known members of the genus \textit{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\textsuperscript{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 \textit{Asanoa}. The isolate could be distinguished from the two known species, \textit{A. ferruginea} and \textit{A. ishikariensis}, by its low relatedness in DNA–DNA hybridization, its cellular fatty acid profile and the utilization of some carbon sources (Table I). On the basis of the data presented above, we propose that strain TT 97-02\textsuperscript{T} be classified in a novel species, \textit{Asanoa iriomotensis} sp. nov.

**Description of \textit{Asanoa iriomotensis} sp. nov.**

\textit{Asanoa iriomotensis} (i.r.i.o.mo.ten’sis. N.L. fem. adj. \textit{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...
Table 1. Diagnostic characteristics that differentiate Asanoa iriomotensis sp. nov. TT 97-02T from other members of the genus Asanoa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain TT 97-02T</th>
<th>A. ferruginea</th>
<th>A. ishikariensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utilization of carbohydrates:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adonitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(+)-D-Lactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(+)-L-Arabinose</td>
<td>W</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major cellular fatty acids</td>
<td>anteiso-C15:0, anteiso-C17:0, iso-C15:0, C17:0, iso-C16:0</td>
<td>anteiso-C15:0, C17:0, iso-C15:0, C17:0, iso-C16:0</td>
<td>anteiso-C15:0, C17:0</td>
</tr>
</tbody>
</table>

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

(−)-D-mannitol, (+)-D-melibiose, (+)-D-maltose, (+)-L-rhamnose, methyl α-D-glucoside, (+)-D-raffinose, (+)-D-galactose, (+)-x-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-C15:0, anteiso-C17:0, iso-C15:0, C17:0 and iso-C16:0. The G+C content of genomic DNA of the type strain is 69 mol%. Habitat is soil.

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

Acknowledgements

The authors acknowledge a Grant-in-Aid for Scientific Research (C) (2) no. 11660326 from the Japan Society for the Promotion of Science. The authors are grateful to Drs Akira Nakagiri, Yasuyoshi Nakagawa, Izumi Okane and Kumiko Ueda-Nishimura for kind help.

References


