Asanoa hainanensis sp. nov., isolated from rhizosphere soil of Acrostichum speciosum in a mangrove, and emended description of the genus Asanoa

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A Gram-reaction-positive, non-motile actinobacterium, designated strain 210121T, was isolated from rhizosphere soil of the mangrove fern Acrostichum speciosum. 16S rRNA gene sequence analysis showed that the isolate belonged to the genus Asanoa. DNA–DNA relatedness values between strain 210121T and the type strains of the three recognized species of the genus Asanoa were below the 70 % threshold recommended for distinguishing bacterial genomic species. The novel isolate contained glutamic acid, glycine, alanine and meso-A2pm as cell-wall amino acids, indicating peptidoglycan type A1γ. The characteristic whole-cell sugars were xylose, ribose, glucose and mannose. The predominant menaquinones were MK-9(H4), MK-9(H6) and MK-9(H8). The major fatty acids were iso-C16 : 0 (30.9 %), C17 : 0 (23.0 %), anteiso-C15 : 0 (14.9 %) and iso-C15 : 0 (12.3 %). The phospholipid profile comprised phosphatidylethanolamine, phosphatidylinositol mannosides and phospholipids of unknown structure containing glucosamine. The G+C content of the DNA was 70.3 mol%. On the basis of the phenotypic and genotypic data, strain 210121T (=CGMCC 4.5593T =DSM 45427T) represents a novel species of the genus Asanoa, for which the name Asanoa hainanensis sp. nov., is proposed. An emended description of the genus Asanoa is also proposed.

The genus Asanoa belongs to the family Micromonomosporaceae and, at the time of writing, contained three species: Asanoa ferruginea (basonym Catellatospora ferruginea; Asano and Kawamoto, 1986), Asanoa ishikariensis (formerly Catellatospora ishikariensis; Lee & Hah, 2002) and Asanoa iriomotensis. A. iriomotensis was isolated from rhizosphere soil of an oriental mangrove tree, Bruguiera gymnorrhiza (Tamura & Sakane, 2005). The rhizosphere of mangroves has proven to be a good source of novel actinobacteria (Hatano, 1997; Takeuchi & Hatano, 1998, 1999; Wang et al., 2011).

Strain 210121T was isolated from a rhizosphere soil sample of the mangrove fern Acrostichum speciosum collected in Hainan Province, China, (19° 37.528’ N 110° 47.601’ E), during an investigation of actinobacteria in mangroves (Hong et al., 2009). A 1 g sample of soil was heated in a hot air oven at 100 °C for 60 min and diluted × 10−2 with quarter-strength Ringer’s solution (King & Hurst, 1963) before sonicating for 10 min in order to disperse the soil (Janssen et al., 2002). Then 100 μl volumes of the suspension were inoculated on to agar plates of glucose-asparagine-vitamin medium (Takahashi et al., 1996) supplemented with cycloheximide (50 mg l−1), nystatin (50 mg l−1) and potassium bichromate (100 mg l−1). After 21 days of aerobic incubation at 28 °C, the isolate, which formed little orange colonies, was transferred and purified on yeast extract–malt extract (International Streptomyces Project, ISP 2) agar (Shirling & Gottlieb, 1966) and maintained as slopes at 4 °C for short-term preservation and as glycerol suspensions (20%, v/v) at −20 °C for long-term preservation (Wellington & Williams, 1978).

Morphological characteristics were observed using light microscopy (80i, Nikon) and scanning electron microscopy.
Biomass for use in chemotaxonomic analyses was obtained from cultures grown in ISP 2 broth on a rotary shaker (~180 r.p.m.) at 28 °C for 4 days. Amino acids and sugars in whole-cell hydrolysates were analysed according to the procedure of Lechevalier & Lechevalier (1980). Analysis of the phospholipids was carried out using TLC according to the procedure of Lechevalier & Lechevalier (1980). Analysis of the peptidoglycan was determined by the method of Sasser (1990) and the composition was obtained for strain 210121T. 16S rRNA gene sequence similarity values between strain 210121T and these three type strains were 98.5, 98.8 and 98.8 % with A. ferruginea IMSNU 22009T, A. ishikariensis IMSNU 22004T and A. iriomotensis TT 97-02T, respectively. DNA–DNA hybridization values between strain 210121T and type strains of species of the genus Asanoa were measured on nylon membranes using the method described by Wang et al. (2011). An almost-complete 16S rRNA gene sequence (1486 nt) was obtained for strain 210121T. 16S rRNA gene sequence analyses revealed that the isolate clustered within the family Micromonosporaceae and belonged to the genus Asanoa (Fig. 1 and Supplementary Fig. S1), sharing sequence similarity values of 98.5, 98.8 and 98.8 % with A. ferruginea IMSNU 22009T, A. ishikariensis IMSNU 22004T and A. iriomotensis TT 97-02T, respectively. DNA–DNA relatedness values between strain 210121T and these three type strains were 55.7 ± 4.9, 44.1 ± 5.6 and 41.8 ± 6.6 %, respectively. The highest 16S rRNA gene sequence similarity value between strain 210121T and type species of other genera in the family Micromonosporaceae was 97.96 % with Micromonospora auratirinigna TT1-11T ( EzTaxon). Cells of strain 210121T were Gram-reaction-positive and aerobic and formed well-developed and branched

Fig. 1. Neighbour-joining phylogenetic tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences (1448 nt) showing the relationships between strain 210121T, the three type strains of species of the genus Asanoa and phylogenetically related members of the family Micromonosporaceae. Phytohabitans sulfiscus K07-0523T was used as an outgroup. Asterisks indicate branches that were also found using the maximum-parsimony (Kluge & Farris, 1969) method. Numbers at branch points indicate bootstrap values >50 % (based on 1000 replicates). Bar, 0.002 substitutions per nucleotide position.
substrate hyphae on ISP 2 medium; no aerial hyphae were produced. The strain grew well on ISP 2, ISP 3, M8 (Castiglione et al., 2008) and ATCC 172 media, moderately well on ISP 1 medium and weakly on ISP 4 and ISP 7 media but did not grow on ISP 6 medium. Strain 210121T could be clearly distinguished from species belonging to the genus Asanoa by their morphological characteristics when cultured on ISP 3 and ATCC 172 media (Table 1). 14-day-old cultures grown on ISP 3 agar medium showed well-developed and branched substrate hyphae but spore chains were not found. The isolate occasionally formed spore chains on tap water agar medium and M8 medium (Supplementary Fig. S2, available in IJSEM Online). These morphological characteristics were similar to those of members of the three recognized species of the genus Asanoa (Lee & Hah, 2002; Tamura & Sakane, 2005), which supported its inclusion in the genus Asanoa.

Strain 210121T could hydrolyse starch, reduce nitrate to nitrite, liquefy gelatin, coagulate and peptonize milk and degrade Tweens 20 and 80 but could not produce melanin, urease or hydrogen sulfide. It grew at 20 °C and 37 °C but not at 10 °C or 45 °C. Growth occurred at pH 6–9 (optimum pH 7) and with 0–3% (w/v) NaCl (optimum 0% NaCl). Results of the biochemical tests are shown in the species description and Table 1.

Cell-wall hydrolysates of strain 210121T contained glutamic acid, glycine, alanine and meso-A2pm, indicating peptido-glycan type A1c (Schleifer & Kandler, 1972). Xylose, ribose, glucose and mannose were found as whole-cell sugars but arabinose and rhamnose were not detected. Phosphatidylethanolamine, phosphatidylinositol mannosides and phospholipids of unknown structure containing glucosamine were detected but phosphatidylcholine was not detected, corresponding to phospholipid type IV (Lechevalier et al.,

### Table 1. Diagnostic characteristics that differentiate strain 210121T from members of the genus Asanoa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of colony on ISP 3 medium</td>
<td>Vivid reddish orange</td>
<td>Brilliant orange</td>
<td>Strong greenish yellow</td>
<td>Moderate olive</td>
</tr>
<tr>
<td>Colour of colony on ATCC 172 medium</td>
<td>Brilliant yellow</td>
<td>Vivid yellowish pink</td>
<td>Deep yellowish pink</td>
<td>Strong reddish orange</td>
</tr>
<tr>
<td>Growth at 37 °C</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Milk coagulation and peptonization</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Urease production</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Carbon utilization</td>
<td>Adonitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Dulcitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(+-)-Lactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Inositol</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(+-)-I-Arabinose</td>
<td>w</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(+-)-I-Rhamnose</td>
<td>w</td>
<td>w</td>
<td>w</td>
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<td></td>
<td>Soluble starch</td>
<td>w</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2-Ketogluutaric acid</td>
<td>w</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Malonic acid, pyruvic acid</td>
<td>w</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Nitrogen utilization</td>
<td>l-Arginine</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>l-Histidine</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>l-4-Hydroxyproline</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>l-Methionine</td>
<td>w</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>l-Serine, l-valine</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Characteristic sugars*</td>
<td>Xyl, Rib, Glu, Man</td>
<td>Ara, Rha, Rib, Xyl, Gal, Man, Glu</td>
<td>Ara, Rha, Rib, Xyl, Gal, Man, Glu</td>
<td>Xyl, Man, Gal, Glu</td>
</tr>
<tr>
<td>Menaquinones*</td>
<td>MK-9(H₂, H₈, H₈α)</td>
<td>MK-10(H₈, H₈α)</td>
<td>MK-10(H₈, H₈α)</td>
<td>MK-10(H₈, H₈α)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)*</td>
<td>70.3</td>
<td>71.5</td>
<td>71.1</td>
<td>69</td>
</tr>
</tbody>
</table>

*Data for reference species (2–4) were taken from Asano & Kawamoto (1986), Lee & Hah (2002) and Tamura & Sakane (2005), all other tests were carried out in parallel under the same conditions.
Asanoa hainanensis sp. nov.

1977). The cellular fatty acids were iso-C_{16:0} (30.9%), C_{17:0} (23.0%), anteiso-C_{15:0} (14.9%), iso-C_{15:0} (12.3%), iso-C_{14:0} (7.9%), anteiso-C_{17:0} (4.9%), C_{17:1} 9c (3.2%) and iso-C_{17:0} (3.0%), corresponding to fatty acid type 2d (Kroppenstedt, 1985). The predominant menaquinones were MK-9(H_4) (31.12%), MK-9(H_6) (39.88%) and MK-9(H_8) (14.14%). The acyl type of the cell-wall muramic acid was glycolyl. The DNA G+C content was 73.0 mol%. Previously characterized members of the genus Asanoa possessed phospholipid type II and contained MK-10(H_4, H_8) as the menaquinone profile (Lee & Hah, 2002; Tamura & Sakane, 2005), whereas strain 210121^T possessed phospholipid type IV and contained MK-9(H_4, H_6, H_8) as the menaquinone profile. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain 210121^T belonged to the genus Asanoa. The isolate could be distinguished from the three recognized species in this genus, A. ferruginea, A. ishikariensis and A. iriomotensis, by DNA–DNA relatedness values of below the 70% threshold for the delineation of bacterial species (Wayne et al., 1987). Therefore, strain 210121^T represents a novel species of the genus Asanoa, for which the name Asanoa hainanensis sp. nov. is proposed.

Emended description of the genus Asanoa
Lee and Hah, 2002

The characteristics of the genus are as described by Lee & Hah (2002), with the following changes. Nitrate may be reduced to nitrite. Contains meso-diaminopimelic acid as the characteristic diamino acid in the peptidoglycan. The whole-cell sugars are xylose, mannose and glucose. The predominant menaquinones are MK-10(H_4, H_8) or MK-9(H_4, H_6, H_8). The phospholipid pattern is type PII or PIV. The DNA G+C content is 69–72 mol%.

Description of Asanoa hainanensis sp. nov.

Asanoa hainanensis (hai.nan.en’sis. N.L. fem. adj. hainan-ensis of or pertaining to Hainan, a tropical province of China, from which the mangrove soil sample was collected).

Cells are aerobic, Gram-reaction-positive, non-motile actinobacteria that form extensively branched substrate hyphae. Forms spore chains on tap water agar medium and M8 medium. Hydrolyses starch, reduces nitrate, liquefies gelatin, coagulates and peptonizes milk and degrades Tween 20 and 80 but does not produce melanin, urease or hydrogen sulfide. Grows at 20–37°C but not at 10 or 45°C. Grows at pH 6–9 and in the presence of 0–3% (w/v) NaCl. Utilizes D-fructose, D-glucose, lactose, (+)-D-xylene, D-ribose, D-mannitol, inositol and dulcitol and weakly utilizes (+)-cellobiose, D-galactose, (+)-trehalose, glycerol, L-arginine, (+)-L-ribose, (+)-L-ribose, soluble starch, sucrose, dextrin, maltose, x-ketoglutaric acid, malonic acid and pyruvic acid but does not utilize adonitol, (+)-melezitose, D-sorbitol, L-sorbose, meso-erythritol, hippuric acid, L-malic acid, DL-lactic acid or succinic acid as sole carbon sources. Utilizes L-4-hydroxyproline, L-serine and L-valine and weakly utilizes L-arginine, L-threonine and L-cysteine but does not utilize L-histidine or L-phenylalanine as sole nitrogen sources. Contains glutamic acid, glycine, alanine and meso-A_3P as cell-wall amino acids. Xylose, ribose, glucose and mannose are the whole-cell sugars (peptidoglycan type A1γ). The acyl type of the cell-wall muramic acid is glycolyl. Phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerolphosphorylcholine and phospholipids of unknown structure containing glucosamine are present. The major cellular fatty acids are iso-C_{16:0}, C_{17:0}, anteiso-C_{15:0} and iso-C_{15:0}. The predominant menaquinones are MK-9(H_4), MK-9(H_6) and MK-9(H_8).

The type strain, 210121^T (=CGMCC 4.5593^T =DSM 45427^T), was isolated from rhizosphere soil of the mangrove fern Acrostichum speciosum in Hainan Province, China. The DNA G+C content of the type strain is 70.3 mol%.

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References


