Description of *Lentzea flaviverrucosa* sp. nov. and transfer of the type strain of *Saccharothrix aerocolonigenes* subsp. *staurosporea* to *Lentzea albida*

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A distinct actinomycete isolated from soil was subjected to polyphasic taxonomic analysis. It is demonstrated by comparative 16S rDNA gene sequencing that the organism, designated strain AS 4.0578ᵀ, represents a novel species of the genus *Lentzea*. The phylogenetic results also showed that it formed a monophyletic lineage distinct from the available members of the genera *Lentzea*, *Lechevalieria* and *Saccharothrix*. The organism was distinguished from all the validly described type strains of the genus *Lentzea* by a combination of phenotypic features and DNA–DNA hybridization. It is proposed, therefore, that strain AS 4.0578ᵀ (= JCM 11373ᵀ) be classified in the genus *Lentzea* as *Lentzea flaviverrucosa* sp. nov. In addition, it is proposed that *Saccharothrix aerocolonigenes* subsp. *staurosporea* NRRL 11184ᵀ be transferred to *Lentzea albida* on the basis of phylogenetic analysis, DNA–DNA homology, nucleotide signatures and phenotypic properties.

**Keywords:** *Lentzea flaviverrucosa* sp. nov., polyphasic taxonomy, 16S rDNA sequencing

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**INTRODUCTION**

The genus *Lentzea* (Yassin *et al.*, 1995) was proposed for aerobic actinomycetes that form abundant aerial hyphae that fragment into rod-shaped elements. Lee *et al.* (2000) proposed that *Lentzea albidocapillata*, the type and only species of the genus *Lentzea*, should be transferred to the genus *Saccharothrix*. However, chemotaxonomic, morphological and physiological properties and phylogenetic analysis demonstrated that the genus *Saccharothrix* was heterogeneous (Labeleda *et al.*, 1984; Labeleda, 1986; Labeleda & Lechevalier, 1989; Labeleda & Lyons, 1989; Grund & Kroppenstedt, 1989; Warwick *et al.*, 1994; Kinoshita *et al.*, 1999; Labeleda & Kroppenstedt, 2000). In a recent proposal, Labeleda *et al.* (2001) revived the genus *Lentzea*, created several novel species (*Lentzea albida* NRRL B-24073ᵀ, *Lentzea californiensis* NRRL B-16137ᵀ, *Lentzea violacea* IMSNU 50388ᵀ and *Lentzea waywayandensis* NRRL B-16159ᵀ) and also proposed *Lechevalieria* gen. nov. according to an analysis of chemotaxonomic properties, almost-complete 16S rDNA sequences and diagnostic nucleotide signatures.

The present investigation was designed to establish the taxonomic position of a soil isolate, AS 4.0578ᵀ, that had been invalidly described as ‘*Streptomyces flavoverrucosus*’ on the basis of a few biochemical, morphological and physiological properties (Yan & Deng, 1966). Genotypic and phenotypic data showed that ‘*Streptomyces flavoverrucosus*’ strain AS 4.0578ᵀ should be recognized as a novel species of *Lentzea*, for which the name *Lentzea flaviverrucosa* sp. nov. is proposed. A recent proposal (Labeleda *et al.*, 2001) revealed that several species of *Saccharothrix* had been misclassified, but the type strain of *Saccharothrix aerocolonigenes* subsp. *staurosporea*, NRRL 11184ᵀ, was not mentioned. In this study, physiological, chemotaxonomic and phylogenetic properties, DNA–DNA relatedness and nucleotide signatures in the 16S
rDNA sequence were used to compare this actinomycete to *Lentzea albida* NRRL B-24073<sup>T</sup> in order to clarify its taxonomic position.

**METHODS**

**Micro-organisms and culture conditions.** Strain AS 4.0578<sup>T</sup> was isolated from soil in Wutaishan Mountain, Shanxi Province, China, by Yan & Deng (1966). The isolate was maintained on modified Sauton’s agar (Mordarska et al., 1972) slants at 4 °C and as a glycerol suspension (20%, v/v) at −20 °C. Biomass for chemotaxonomic and molecular-systematic studies was prepared by growing the strain in shake flasks of modified Sauton’s broth at 28 °C for 7 days. Cultures were then checked for purity, harvested by centrifugation, washed twice with distilled water and freeze-dried.

**Cultural and morphological properties.** Gross morphological observations were made by using cultures grown at 28 °C for 14 days on the standard media suggested by the International Streptomycetes Project (ISP) (Shirling & Gottlieb, 1966) and glucose/yeast extract/malt extract (GYM) agar. Melanin pigment production was determined using peptone/yeast extract/iron agar (ISP medium 6) and tyrosine agar (ISP medium 7). Micromorphology was examined using the cover-slip technique. Growth on cover slips was fixed and examined according to the procedures described by Zhou et al. (1998). Additional morphological characters were examined by using a Hitachi S-570 scanning electron microscope.

**Phenotypic tests.** The test strains were examined for a range of phenotypic properties following the procedures of Lee et al. (2000), Yassin et al. (1995) and Gordon et al. (1974). To determine lysozyme sensitivity, a 0.05% (w/v) solution of lysozyme was sterilized by membrane filtration and added to sterilized Sauton’s broth (McCarthy & Cross, 1984) to give a final concentration of 0.0025% (w/v). Test tubes of the basal medium with and without lysozyme were inoculated with fresh cultures and examined for growth after 10 days at either 28 or 50 °C. Antibiotic-sensitivity tests were carried out by placing impregnated filter-paper discs (Goodfellow & Orchard, 1974) over Sauton’s agar and incubating the samples for 7–10 days at the incubation temperatures cited above. Growth was tested at temperatures of 37, 42 and 45 °C. For comparative purposes, *Lentzea violacea* IMNSU 50388<sup>T</sup> and *Lentzea albicolorapillata* NRRL B-24057<sup>T</sup> were used as reference strains.

**Chemotaxonomy.** The isomeric form of diaminopimelic acid (DAP), predominant whole-organism sugars and the phospholipid pattern of the test strain were detected following standard procedures developed by Lechevalier & Lechevalier (1980) and Hasegawa et al. (1985). Menaquinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu et al., 1989), with *Streptomycetes griseus* as the control. Standard TLC protocols were also used for the extraction and analysis of mycolic acids (Minnikin et al., 1980). Fatty acids were extracted, purified by preparative TLC, separated, identified and quantified by GC as described by Yassin (1988).

**DNA base composition and DNA homology analysis.** Genomic DNA was extracted by employing the method of Chun & Goodfellow (1995). The G + C content of the DNA was determined by the thermal denaturation midpoint (T<sub>m</sub>) method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as the control. The DNA homology analysis was carried out by measurement of DNA–DNA hybridization from renaturation rates (De Ley et al., 1970).

**16S rDNA sequencing.** PCR amplification of the 16S rDNA was performed as described by Kim et al. (1996) and the resultant PCR products were purified using the Wizard PCR purification system (Promega) according to the procedures provided by the manufacturer. The purified products were sequenced directly using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) and universal primers (Huang et al., 2001). The nucleotide sequence was obtained automatically by using an Applied Biosystems 373A DNA sequencer according to the manufacturer’s protocols.

**Phylogenetic analysis.** The 16S rDNA sequence of strain AS 4.0578<sup>T</sup> was aligned manually against corresponding sequences of type species of the genera *Saccharothrix*, *Lentzea* and *Lechevalieria* retrieved by BLAST search from EMBL/GenBank using CLUSTAL X 1.8 (Thompson et al., 1997). Phylogenetic trees were inferred by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) treeing algorithms, from the PHYLIP package (Felsenstein, 1993). Evolutionary-distance matrices were generated as described by Kimura (1980). Tree topologies were evaluated by carrying out bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset using the programs seboot and CONSENSE provided in the PHYLIP package (Felsenstein, 1993).

**RESULTS AND DISCUSSION**

An almost-complete 16S rDNA sequence was determined for strain AS 4.0578<sup>T</sup> (1426 nt). Comparison of this sequence with sequences of all validly published type strains of the genera *Lentzea*, *Lechevalieria* and *Saccharothrix* showed that strain AS 4.0578<sup>T</sup>, *Lentzea violacea* IMNSU 50388<sup>T</sup>, *Lentzea albicolorapillata* NRRL B-24057<sup>T</sup>, *Lentzea waywayandensis* NRRL B-16159<sup>T</sup>, *Lentzea albida* NRRL B-24073<sup>T</sup> and *Lentzea californiensis* NRRL B-16137<sup>T</sup> formed a lineage that also contains *Saccharothrix aerocolonigenes* subsp. *staurosporea* NRRL 11184<sup>T</sup> (Fig. 1). Although pairwise comparison of 16S rDNA sequences indicated that strain AS 4.0578<sup>T</sup> shared the highest 16S rDNA similarity with *Lentzea violacea* IMNSU 50388<sup>T</sup> (98.3%), *Lentzea albicolorapillata* NRRL B-24057<sup>T</sup> (97.7%) and *Lentzea waywayandensis* NRRL B-16159<sup>T</sup> (97.5%), strain AS 4.0578<sup>T</sup> showed low DNA–DNA relatedness with *Lentzea violacea* IMNSU 50388<sup>T</sup> (34%), *Lentzea albicolorapillata* NRRL B-24057<sup>T</sup> (43%) and *Lentzea waywayandensis* NRRL B-16159<sup>T</sup> (12%). The 16S rDNA similarity of strain AS 4.0578<sup>T</sup> to the remaining validly published type strains of the genera *Lentzea*, *Lechevalieria* and *Saccharothrix* ranged from 96.6 to 97.4%. *Saccharothrix aerocolonigenes* subsp. *staurosporea* NRRL 11184<sup>T</sup> and its closest neighbour, *Lentzea albida* NRRL B-24073<sup>T</sup>, formed a monophyletic lineage (99-1% sequence similarity) within the genus *Lentzea*, and the level of DNA–DNA hybridization between the two strains (89%) indicated that these two taxa belong to the same species, assuming that species boundaries occur at DNA similarity values of approximately 70%. An
**Lentzea flaviverrucosa** sp. nov.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rDNA sequences showing relationships between **Lentzea flaviverrucosa** sp. nov., **Saccharothrix aerocolonigenes** subsp. **staurosporea** and all the validly described species of the genera **Lentzea**, **Lechevalieria** and **Saccharothrix**. f and m indicate branches that were also recovered when the Fitch–Margoliash and maximum-likelihood methods were used. Numbers at nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values over 50% are given. Bar, 0.01 substitutions per nucleotide position.

Examination of the aligned sequences for the 16S rDNA gene (Fig. 2) revealed that the diagnostic nucleotide signatures (TCCA, positions 617–620; GCC, 843–845) of strain AS 4.0578\(^T\) and **Saccharothrix aerocolonigenes** subsp. **staurosporea** and all the validly described species of the genera **Lentzea**, **Lechevalieria** and **Saccharothrix**. f and m indicate branches that were also recovered when the Fitch–Margoliash and maximum-likelihood methods were used. Numbers at nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values over 50% are given. Bar, 0.01 substitutions per nucleotide position.

Fig. 2. Signatures in the 16S rDNA sequences of **Lentzea flaviverrucosa** sp. nov., **Saccharothrix aerocolonigenes** subsp. **staurosporea** and representatives of the genera **Lentzea**, **Lechevalieria** and **Saccharothrix**.

Fig. 3. Scanning electron micrograph showing rod-shaped fragments of the aerial mycelium of strain AS 4.0578\(^T\) grown on modified Sauton’s agar for 8 days at 28°C. Magnification × 8000.

**Lentzea waywaywendenis** NRRL B-16159\(^T\) (AF114813)

**Saccharothrix aerocolonigenes** subsp. ** staurosporea** NRRL B-16137\(^T\) (AF174435)

**Lentzea flaviverrucosa** AS 4.0578\(^T\) (AF183957)

**Lentzea albiscapillata** NRRL B-24057\(^T\) (X84321)

**Saccharothrix violacea** IMSNU 50388\(^T\) (A242633)

**Lentzea waywaywendenis** NRRL B-16115\(^T\) (AF114805)

**Saccharothrix syringae** NRRL B-16486\(^T\) (AF114812)

**Saccharothrix violacea** IMSNU 50388\(^T\) (A242633)

**Lentzea waywaywendenis** NRRL B-16115\(^T\) (AF114805)

**Saccharothrix syringae** NRRL B-16486\(^T\) (AF114812)

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11184\(^T\) were in agreement with the observation of Labeda et al. (2001) regarding this genus.

Morphologically, strain AS 4.0578\(^T\) was quite similar to all species of **Lentzea**. The vegetative hyphae were well developed, with moderate, irregular branching, and penetrated the agar, forming yellowish, verrucose colonies on the agar surface. Substrate mycelium is pale yellow to yellowish brown on ISP media 2, 3 and 4 and GYM agar. The aerial mycelium was well developed on Sauton’s agar and fragmented into rod-shaped elements (Fig. 3). Sparse, white to yellowish white aerial mycelium is produced on oatmeal agar or GYM agar. **Saccharothrix aerocolonigenes** subsp. **staurosporea** NRRL B-24073\(^T\) according to Labeda et al. (2001) and Takahashi et al. (1995). The physiological properties that differentiate strain AS 4.0578\(^T\), **Saccharothrix aerocolonigenes** subsp. **staurosporea** NRRL 11184\(^T\) also shared similar morphological properties with **Lentzea albida** NRRL B-24073\(^T\) according to Labeda et al. (2001) and Takahashi et al. (1995). The physiological properties that differentiate strain AS 4.0578\(^T\), **Saccharothrix aerocolonigenes** subsp. **staurosporea** NRRL 11184\(^T\)
Table 1. Differential physiological properties of Lentzea species

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* Data from Takahashi et al. (1995).

and all described species of Lentzea are shown in Table 1.

The chemotaxonomic properties of strain AS 4.0578° and Saccharothrix aerocolonigenes subsp. stauroporea NRRL 11184° were also consistent with those of the genus Lentzea (Table 2). The two organisms have been observed to possess chemical characteristics typical of the genus Lentzea (containing galactose, mannose and ribose; lacking tuberculostearic acid in fatty acid profiles; lacking hydroxyphosphatidyl ethanolamine) rather than the genus Saccharothrix, the members of which contain only trace amounts of mannose as a diagnostic whole-cell sugar and may contain hydroxyphosphatidyl ethanolamine.

According to the data mentioned above, it is proposed that strain AS 4.0578° be classified as a novel member of the genus Lentzea, Lentzea flaviverrucosa sp. nov., and that Saccharothrix aerocolonigenes subsp. stauroporea NRRL 11184° be validly transferred to Lentzea albida.

Description of Lentzea flaviverrucosa sp. nov.

Lentzea flaviverrucosa (f.l.a.vi.ver.ru.co’sa. L. adj. flavus yellowish; L. adj. verrucosus verrucose; N.L. fem. adj. flaviverrucosa yellowish and verrucose, referring to the yellowish, verrucose colony morphology observed on the agar surface).

Gram-positive, non-acid-fast. Substrate mycelium is pale yellow to yellowish brown on ISP media 2, 3 and 4 and GYM agar. Sparse, white to yellowish white aerial mycelium is produced on oatmeal agar and GYM agar. Melanin pigment is not produced on either ISP medium 6 or 7. No soluble pigments are produced. Catalase-positive. Elastin, starch, gelatin,
Table 2. Chemical characteristics of *Lentzea flaviverrucosa* sp. nov. and *Saccharothrix aerocolonigenes* subsp. *staurosporea* compared with the genera *Lechevalieria*, *Lentzea* and *Saccharothrix*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Lechevalieria</em></th>
<th><em>Saccharothrix</em></th>
<th><em>Lentzea</em></th>
<th><em>Lentzea flaviverrucosa</em> AS 4.0578*</th>
<th><em>Saccharothrix aerocolonigenes</em> subsp. <em>staurosporea</em> NRRL 11184*</th>
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<tr>
<td>Whole-cell sugars</td>
<td>Galactose, mannose, rhamnose</td>
<td>Galactose, rhamnose, mannose (trace)</td>
<td>Galactose, mannose, ribose</td>
<td>Galactose, mannose, ribose, glucose</td>
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<tr>
<td>Phospholipids*</td>
<td>PE</td>
<td>PE, OH-PE, PI, PIMs, DPG, PG</td>
<td>PE, DPG, PG, PI</td>
<td>PE, DPG, PIMs</td>
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<tr>
<td>Predominant menaquinones</td>
<td>MK-9(H₄)</td>
<td>MK-9(H₄)</td>
<td>MK-9(H₄)</td>
<td>MK-9(H₄), MK-9(H₄)</td>
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</table>

* DPG, Diphosphatidyl glycerol; OH-PE, hydroxyphosphatidyl ethanolamine; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PIMs, phosphatidyl inositol mannosides.

hypoxyanthine and tyrosine are hydrolysed or decomposed, but casein, adenine, aesculin, allantoin, quanine, xanthine, urea and chitin are not. Nitrate is reduced to nitrite. Phosphtase and lipase are produced. Growth occurs in the presence of 1 and 1.5% NaCl but not in the presence of 2 or 4% NaCl. Assimilates acetate, adipate, malate, malonate, propionate and pyruvate, but not benzoate, oxalate, citrate, formate, lactate, sebacic acid, succinate or tartrate. Acid is produced from citrate, formate, lactate, sebacic acid, succinate or pionate and pyruvate, but not benzoate, oxalate, citrate, formate, lactate, sebacic acid, succinate or tartrate. Acid is produced from D-cellulose, dextrin, *meso*-erythritol, glyceral, *meso*-inositol, maltose, mannotol, mannosse, melibiose, L-raffinose, salicin and D-sucrose, but not from adonitol, L-arabinose, lactose, L-rhamnose, D-sorbitol, D-xylose or methyl α-D-glucoside. Grows at 37 and 42 °C but not at 45 °C. Growth is inhibited by lysozyme (0.0025%, w/v), amikacin (disks soaked in 30 μg antibiotic ml⁻¹), amoxicillin plus clavulanic acid (10 μg ml⁻¹), cefotaxime (30 μg ml⁻¹), ciprofloxacin (5 μg ml⁻¹), clindamycin (2 μg ml⁻¹), gentamicin (15 μg ml⁻¹), kanamycin (30 μg ml⁻¹), mezlocillin (75 μg ml⁻¹), novobiocin (5 μg ml⁻¹), ofloxacin (5 μg ml⁻¹) and tetracycline (30 μg ml⁻¹), but not by ampicillin (10 μg ml⁻¹), aztreonam (30 μg ml⁻¹), chloromycine (30 μg ml⁻¹), erythromycin (15 μg ml⁻¹) or tobramycin (10 μg ml⁻¹). The cell wall is of type III (*meso*-DAP). The whole-cell sugar pattern consists of galactose, mannosae, glucose and ribose. Possesses the type II phospholipid pattern (phosphatidyl ethanolamine, diphostatidyl glycerol and phosphatidylinositol mannosides). The major menaquinones are MK-9(H₄) and MK-9(H₄) (peak area ratio 31:23). Mycolic acids are absent. The predominant fatty acid is i-C₁₆:₀ (35-51% of total) and considerable amounts of C₁₆:₁ (19-51%), i-C₁₅:₀ (9-52%), C₁₇:₀ (6-74%), 10Me-C₁₆:₀ (6-13%) and a-C₁₅:₀ (5-18%) are also present. The G+C content of the DNA is 64.1 mol% (Tₘ). Isolated from a soil sample collected in Wutaishan Mountain, Shanxi Province, China. The type strain is strain AS 4.0578* (= JCM 11373*).

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**REFERENCES**


