Revival of the genus Lentzea and proposal for Lechevalieria gen. nov.

D. P. Labeda, K. Hatano, R. M. Kroppenstedt and T. Tamura

The genus Saccharothrix is phylogenetically heterogeneous on the basis of analysis of almost complete 16S rDNA sequences. An evaluation of chemotaxonomic, morphological and physiological properties in the light of the molecular phylogeny data revealed that several species are misclassified. Saccharothrix aerocolonigenes NRRL B-3298T and Saccharothrix flava NRRL B-16131T constitute a lineage distinct from Saccharothrix and separate from Lentzea. The genus Lechevalieria gen. nov. is proposed for these species. Lechevalieria aerocolonigenes comb. nov. is the type species and S. flava is transferred as Lechevalieria flava comb. nov. Although Lentzea albido-capillata, the type species of the genus Lentzea, was transferred recently to the genus Saccharothrix, the revival of Lentzea is clearly supported by molecular phylogenetic and chemotaxonomic data. The description of the revived genus is emended to include galactose, mannose and traces of ribose as diagnostic whole-cell sugars and MK-9(H4) as the principal menaquinone and elimination of tuberculostearic acid as a diagnostic component in the fatty acid profile. Saccharothrix waywayandensis NRRL B-16159T, S. aerocolonigenes NRRL B-16137 and ’Asiosporangium albidum’ IFO 16102 are members of the amended genus Lentzea on the basis of phylogenetic and chemotaxonomic properties. S. waywayandensis is transferred to Lentzea as Lentzea waywayandensis comb. nov., while the new species Lentzea californiensis sp. nov. and Lentzea albida sp. nov. are described for S. aerocolonigenes NRRL B-16137 and ‘A. albidum’ IFO 16102, respectively. Nucleotide signatures in the 16S rDNA sequences are defined that are diagnostic for the genera Lechevalieria, Lentzea and Saccharothrix.

Keywords: Pseudonocardineae, Actinosynnemataceae, Saccharothrix

INTRODUCTION

A phylogenetic analysis of species of the genus Saccharothrix and allied taxa within the family Actinosynnemataceae, based on partial 16S rDNA sequences (Labeda & Kroppenstedt, 2000), demonstrated that Saccharothrix is phylogenetically heterogeneous and that several of the described species are misclassified. It was noted that Saccharothrix waywayandensis NRRL B-165159T is more closely related phylogenetically to Lentzea albido-capillata than to Saccharothrix sensu stricto and that Saccharothrix aerocolonigenes NRRL B-3298T and Saccharothrix flava NRRL B-16131T form a lineage distinct from Saccharothrix and separate from Lentzea. The previous study of Tamura & Hatano (1998) also demonstrated that ‘Asiosporangium albidum’ IFO 16102 is phylogenetically related to Saccharothrix and Lentzea. A recent proposal (Lee et al., 2000) suggested that Lentzea albido-capillata, the type species of the genus Lentzea, be transferred into the genus Saccharothrix, thus effectively abolishing this genus under the rules of the Bacteriological Code of Nomenclature (Lapage et al., 1992), but the phylogenetic and chemotaxonomic data in the present study do not support this proposal. An evaluation of the chemotaxonomic, morphological and physiological properties of these actinomycetes was undertaken to clarify their taxonomic position.
METHODOLOGICAL METHODS

Strains, cultivation and maintenance. The strains evaluated in the present study are listed in Fig. 1. Primary storage of strains was as lyophilized ampoules of mycelial and spore suspensions in sterile beef serum held at 4 °C. Working stock cultures were maintained on slants of ATCC medium no. 172 (Cote et al., 1984) and stored at 4 °C until needed. Biomass for extraction of DNA was grown as 7 d streak cultures on ATCC medium no. 172 agar plates.

Chemotaxonomic analysis of strains for menaquinones, fatty acids and whole-cell sugars was performed using methods described previously (Grund & Kroppenstedt, 1989).

Physiological tests. Physiological tests, including those for the production of acid from carbohydrates, the utilization of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, ascorbic acid, urea and hippurate, were evaluated by using the method of Kurup & Schmitt (1973). Phosphatase activity was evaluated by using the method of Gordon et al. (1974) for aesculin hydrolysis. Phosphatase activity was evaluated by using the method of Gordon et al. (1974) for aesculin hydrolysis. Phosphatase activity was evaluated by using the method of Kurup & Schmitt (1973). The temperature range for growth was determined on slants of ATCC medium no. 172 agar (Cote et al., 1984).

DNA isolation, 16S rDNA gene amplification and sequencing. Genomic DNA was isolated, purified and sequenced using procedures described previously (Labeda & Kroppenstedt, 2000).

Results and Discussion

The neighbour-joining dendrogram for the family Actinosynnemataceae, resulting from phylogenetic analysis of 16S rDNA sequences, is shown in Fig. 1; it can be clearly observed that the genus Saccharothrix sensu lato exhibits phylogenetic heterogeneity upon analysis based on almost complete sequences of 16S rDNA. Similar tree topographies resulted from analy-
Les strains using the maximum-parsimony and maximum-
likelihood algorithms (trees not shown). The phylo-
genetic position of *Saccharothrix cryophilis* NRRL B-
16238 indicates that this species does not belong in
the genus *Saccharothrix* and that it probably does not
belong within the family *Actinosynnemataceae*, but
this will be the subject of another detailed paper.
*S. aerocolonigenes* NRRL B-16137, *Saccharothrix
violacea* IMNSU 50388, *S. waywayandensis* NRRL B-
16159 and ‘*A. albidum*’ IFO 16102 form a lineage
that contains *Lentzea albidocapillata* DSM 44073
(97.8% mean intrageneric nucleotide similarity), the
type species of the genus, which is also clearly
separated phylogenetically from *Actinosynnema
violacea* and *S. flava*. These strains also
propose that they be transferred to the revived and
emended genus *Lentzea*.

The type strains of *S. aerocolonigenes* and *S. flava*,
respectively NRRL B-3298T and NRRL B-16131T,
consistently formed a monophyletic lineage distinct
from *Saccharothrix* (mean nucleotide similarity of
96.8% to species of this genus) and intermediate
between *Lentzea* (mean nucleotide similarity of 96.7%
to species of this genus) and *Actinosynnema* (mean
nucleotide similarity of 97.3% to species of this genus)
for all of the algorithms tested. The phylogenetic
validity of the genera *Actinosynnema*, *Lentzea* and
*Saccharothrix* was consistently well supported stat-
istically by all algorithms used; the *S. aerocolonigenes*
and *S. flava* lineage was consistently found between
*Actinosynnema* and *Lentzea*, regardless of which
algorithm was used. An examination of the aligned
sequences for the 16S rDNA gene (Fig. 2) revealed that
diagnostic nucleotide signatures can be used to
differentiate the *S. aerocolonigenes–S. flava* lineage
from *Lentzea* and *Actinosynnema* and that they can
also be used to differentiate them from the other
genera of the *Actinosynnemataceae*. Although *S.
aerocolonigenes* and *S. flava* appear to be phylogenetically
close to the genus *Actinosynnema*, neither has been
observed to produce coremia with chains of motile
spores (this feature being characteristic of this genus).
It is therefore proposed that the *S. aerocolonigenes–S.
flava* lineage represents a new genus, to be called
*Lentzea* gen. nov.

Analysis of the chemotaxonomic characteristics of
*Lentzea albidocapillata* DSM 44073 in the present
study revealed the presence of galactose, mannose and
ribose as diagnostic sugars and menaquinone MK-
9(H$_9$) as the predominant menaquinone and the
absence of any significant amount of 10-methyl C18:0
fatty acids (tuberculostearic acid). This is largely in
agreement with the observations of Lee et al. (2000)
regarding this strain. Galactose and mannose were
found as diagnostic sugars in whole-cell hydrolysates
of all strains examined, but ‘*A. albidum*’ IFO 16102
and *S. aerocolonigenes* NRRL B-16137 contained
ribose, while *S. waywayandensis* NRRL B-16159T,
*S. aerocolonigenes* NRRL B-3298T and *S. flava* NRRL B-
16131T did not. Whilst the genera *Lechevalieria* and
*Lentzea* (including ‘*A. albidum*’, *S. aerocolonigenes*
NRRL B-16137 and *S. waywayandensis* NRRL B-
16159T) share many diagnostic chemotaxonomic
characteristics with *Saccharothrix*, they can be
distinguished from this genus by the lack of hydroxy-
substituted fatty acids in the phosphatidyl ethanol-
amine component of their phospholipids (Table 1).
*Saccharothrix* and *Lechevalieria* strains tend to contain
varying amounts of rhamnose in whole-cell hydro-
lysates, whereas *Lentzea* strains were observed to lack
(or contain only trace amounts of) rhamnose, and
might also contain ribose.

All species of *Lentzea* produce branching substrate
mycelium, and the aerial mycelium fragments into rod-
shaped elements, although *Lentzea waywayandensis*
Table 1. Chemotaxonomic characteristics of *Lechevalieria* compared with other genera of the family *Actinosynnemataceae*

All of the genera have *meso*-diaminopimelic acid as the cell wall diamino acid, are of cell wall chemotype III and contain straight-chain, mono-unsaturated, iso and anteiso fatty acids. Abbreviations: DPG, diphosphatidyl glycerol; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PIMs, phosphatidyl inositol mannosides; PME, phosphatidyl methylethanolamine.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Actinokineospora</th>
<th>Actinosynnema</th>
<th>Asiosporangium</th>
<th>Lechevalieria</th>
<th>Lentzea</th>
<th>Saccharothrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-cell sugar pattern</td>
<td>Galactose, mannose, rhamnose</td>
<td>Galactose, mannose</td>
<td>Galactose, mannose, ribose</td>
<td>Galactose, mannose, rhamnose</td>
<td>Galactose, mannose, ribose</td>
<td>Galactose, rhamnose, mannose (trace)</td>
</tr>
<tr>
<td>Phospholipid type</td>
<td>PII</td>
<td>PII</td>
<td>PII</td>
<td>PII</td>
<td>PII, PIV</td>
<td>PII</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>PE, OH-PE</td>
<td>PE, OH-PE, PI, PIMs, DPG</td>
<td>PE, OH-PE, PI, PIMs, DPG, PG</td>
<td>PE</td>
<td>PE</td>
<td>PE, OH-PE, PI, PIMs, DPG, PG</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>MK-9(H₉)</td>
<td>MK-9(H₉), MK-9(H₉)</td>
<td>MK-9(H₉)</td>
<td>MK-9(H₉)</td>
<td>MK-9(H₉)</td>
<td>MK-9(H₉), MK-9(H₉)</td>
</tr>
</tbody>
</table>

Table 2. Differential physiological properties of *Lentzea* species

w, Weak positive reaction. Data for *Lentzea violacea* were taken from Lee *et al.* (2000).

<table>
<thead>
<tr>
<th>Property</th>
<th><em>Lentzea albidocapillata</em> NRRL B-24057&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Lentzea albida</em> NRRL B-24073&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Lentzea californiensis</em> NRRL B-16137&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Lentzea violacea</em> IMSNU 50388&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Lentzea waywayandensis</em> NRRL B-16159&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of urea</td>
<td>w</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reductase production</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Assimilation of:</td>
<td></td>
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</tr>
<tr>
<td>Acetate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Malate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Production of acid from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adonitol</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>37 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42 °C</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>45 °C</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NRRL B-16159<sup>T</sup> produces only relatively sparse aerial mycelium in comparison with the other *Lentzea* species. Both species of *Lechevalieria* produce very sparse aerial mycelium on agar media and have not been observed to produce sporangia, coremia or motile spores, which differentiates them from *Actinokineospora* and *Actinosynnema*.

It is proposed that the formal description of the revived genus *Lentzea* be emended to include updated chemotaxonomic data from both the present study and that of Lee *et al.* (2000). On the basis of the data presented, *S. violacea* is transferred as *Lentzea violacea* comb. nov., *S. waywayandensis* is transferred as *Lentzea waywayandensis* comb. nov. and ‘*A. albidum*’ IFO 16102 is validly described as *Lentzea albida* sp. nov. Because it is proposed that the type strain of *S. aerocolonigenes* be transferred to the new genus *Lechevalieria* and as strain NRRL B-16137 is a member of the genus *Lentzea*, it is described as the new species *Lentzea californiensis* sp. nov. The physiological properties that differentiate these species are shown in Table 2 and the formal descriptions are given below.
The physiological properties that differentiate the *Lechevalieria* species can be seen in Table 3 and the formal descriptions of the genus *Lechevalieria* and the species *Lechevalieria aerocolonigenes* and *Lechevalieria flava* are also given below.

**Emended description of Lentzea nom. rev., emend.**

The formal description of Yassin et al. (1995) is emended to reflect the following changes in the chemotaxonomic characteristics. The diagnostic whole-cell sugars include galactose and mannose and might also include ribose. The major menaquinone is MK-9(H$_4$). Tuberculostearic acid is found only in trace quantities, if at all, in fatty acid profiles. The sequence of the 16S rRNA gene contains a genus-specific diagnostic nucleotide signature pattern, namely TCCA (617–620) and GCC (843–845).

**Description of Lentzea albida sp. nov.**

*Lentzea albida* (al’bi.da. L. fem. adj. albida whitish, referring to the colour of the aerial mycelium).

Substrate mycelium is a yellowish-orange in colour on most media. Copious white aerial mycelium is produced. Soluble pigments are not produced. Casein, aesculin, hypoxanthine, starch and tyrosine are hydrolysed. No hydrolysis of adenine, allantoin or xanthine. Growth occurs in the presence of 4 and 5% NaCl. Assimilates acetate, citrate, malate, propionate and succinate. Does not assimilate benzoate, lactate, mucate, oxalate or tartrate. Acid is produced from adonitol, dulcitol, erythritol, methyl β-xyloside or sorbitol. Grows at 37 °C but not at 42 °C. Isolated from a soil sample from California. Strain NRRL B-16137 was originally classified as *Saccharothrix aerocolonigenes*. The type strain of *Lentzea californiensis* is NRRL B-16137$^T$ (= DSM 43393$^T$ = IMRU 550$^T$).

**Description of Lentzea californiensis sp. nov.**

*Lentzea californiensis* (cal.i.for.ni.en’sis. N.L. gen. n. *californiensis* of California, referring to the source of this isolate, soil from California).

Substrate mycelium is yellow to orange-brown. White aerial mycelium is produced. Orange, soluble pigments may be produced on Czapek’s agar. Casein, aesculin, hypoxanthine, starch and tyrosine are hydrolysed. Does not hydrolyse adenine, allantoin or xanthine. Grows in the presence of 5% NaCl. Assimilates citrate, malate, propionate and succinate. Does not assimilate acetate, benzoate, lactate, mucate, oxalate or tartrate. Acid is produced from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannosone, melibiose, raffinose, rhamnose, sucrose, trehalose and xylose; acid is not produced from adonitol, dulcitol, erythritol, methyl β-xyloside or sorbitol. Grows at 37 °C but not at 42 °C. Isolated from a soil sample from California.

**Description of Lentzea violacea comb. nov.**

Basonym *Saccharothrix violacea* Lee et al. 2000.

The morphological and physiological properties of this species have been described previously (Lee et al., 2000). The type strain of *Lentzea violacea* is IMSNU 50388$^T$.

**Description of Lentzea waywayandensis comb. nov.**


The morphological and physiological properties of this species have been described previously (Labeda & Lyons, 1989). The type strain of *Lentzea waywayandensis* is NRRL B-16159$^T$ (= DSM 44232$^T$ = IFO 14970$^T$).

**Description of Lechevalieria gen. nov.**

*Lechevalieria* (Le.che.val.i.er’i.a. N.L. fem. n. *Lechevalieria* of Lechevalier, named after the American microbiologists Hubert and Mary Lechevalier, who contributed substantially to the field of actinomycete biology during their careers at the Waksman Institute of Microbiology).

Branching vegetative mycelium (approx. 0.5 µm in diameter) is produced. Very scant aerial mycelium is produced on some media. Gram-positive. Lysozyme-resistant. Catalase-positive and aerobic. The cell wall...
is of type III (meso-diaminopimelic acid). The whole-cell sugar pattern consists of galactose, mannose and traces of rhamnose. Possesses the type PII phospholipid pattern, with significant quantities of phosphatidyl ethanolamine lacking hydroxylated fatty acids. The predominant menaquinone is MK-9(H4). The fatty acid profile consists of saturated and mono-unsaturated iso and anteiso fatty acids. Phylogenetically, the genus represents a line of descent in the Actinosynemataceae adjacent to the genus Saccharothrix and close to the genera Actinosynema and Lentzea. The sequence of the 16S rRNA gene contains a genus-specific diagnostic nucleotide signature pattern, namely TT (844–845) and GGT (1107–1109). The type species is Lechevalieria aerocolonigenes.

**Description of Lechevalieria aerocolonigenes comb. nov.**


The morphological and physiological properties of this species have been described previously (Labeda, 1986). The type strain of *Lechevalieria aerocolonigenes* is NRRL B-3298T (= ATCC 23870T = CCRC 13661T = DSM 40034T = IFO 13195T = JCM 4150T).

**Description of Lechevalieria flava comb. nov.**


The morphological and physiological properties of this species have been described previously (Grund and Kroppenstedt, 1989). The type strain of *Lechevalieria flava* is NRRL B-16131T (= ATCC 29533T = CCRC 13328T = DSM 43885T = IFO 14521T = INA 2171T = JCM 3296T).

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**REFERENCES**


