**Crossiella gen. nov., a new genus related to Streptoalloteichus**

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Phylogenetic analysis of the genera within the suborder Pseudonocardineae based on almost complete sequences of 16S rDNA showed that *Saccharothrix cryophilis* NRRL B-16238T was misplaced within the genus *Saccharothrix*. *Saccharothrix cryophilis* NRRL B-16238T appeared to be phylogenetically closest to *Streptoalloteichus*, but is morphologically distinct from this genus because sporangia with motile spores are not observed. The aerial mycelium fragments into rod-shaped elements and sclerotium-like bodies are observed occasionally in the substrate mycelium. The cell wall contains meso-diaminopimelic acid, whole-cell hydrolysates contain galactose, rhamnose and ribose, the phospholipid pattern is type PIV and the principal menaquinone is MK-9(H4). A new genus to accommodate *Saccharothrix cryophilis* is proposed, *Crossiella* gen. nov., in recognition of the contributions of Thomas Cross, a distinguished actinomycete biologist at the University of Bradford, UK. The type species is *Crossiella cryophila* gen. nov., comb. nov.

**Keywords:** Pseudonocardineae, Actinosynnemataceae, polyphasic taxonomy, Saccharothrix

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**INTRODUCTION**

‘*Nocardiopsis mutabilis* subsp. *cryophilis*’ was described by Takahashi *et al.* (1986) for a novel soil isolate that produced the antibiotic dopsamine. The authors noted that this strain exhibited some morphological characteristics that were different from members of the genus *Nocardiopsis* but, based on chemotaxonomic properties, they felt that this genus was the closest fit. It was subsequently proposed in two independent studies that *Nocardiopsis mutabilis* be transferred to the genus *Saccharothrix* (Grund & Kroppenstedt, 1989; Labeda & Lechevalier, 1989), and an evaluation of DNA relatedness between the type strain of ‘*Nocardiopsis mutabilis* subsp. *cryophilis*’, NRRL B-16238T, and the other taxa of the genus *Saccharothrix* demonstrated that this strain represented a distinct species within *Saccharothrix* (Labeda & Lechevalier, 1989). A recent phylogenetic study of *Saccharothrix* and related genera based on almost complete 16S rDNA sequences resulted in the creation of the new family Actinosynnemataceae (Labeda & Kroppenstedt, 2000), as well as some taxonomic reorganization among species included within the genus *Saccharothrix* (Labeda *et al.*, 2001). This phylogenetic analysis indicated strongly that *Saccharothrix cryophilis* NRRL B-16238T was not a member of the genus *Saccharothrix*, or even of the family Actinosynnemataceae, but exhibited a close relationship to the genus *Streptoalloteichus*. A polyphasic study was undertaken to confirm and expand on the published characteristics of this strain in support of the proposal to transfer it to a new genus within the suborder Pseudonocardineae (Stackebrandt *et al.*, 1997).

**METHODS**

**Strains, cultivation and maintenance.** 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 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 172 agar plates.

**Morphological observations.** Gross morphological observations were made using cultures grown for 14 d at 28 °C on the standard media suggested by the International Streptomyces Project (Shirling & Gottlieb, 1966) and Czapek’s sucrose agar (Pridham & Lyons, 1980). Micromorphology and sporulation were observed by light microscopy and scanning electron microscopy (SEM). Samples for SEM observation were 14-d cultures on agar media fixed overnight.
with osmium tetroxide vapours, post-fixed for 1 h in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.3, dehydrated through a graded acetone series and then critical-point dried from liquid CO₂ and sputter-coated with gold/palladium. The samples were observed using a JEOL model JSM 6400 V scanning electron microscope.

Chemotaxonomy. 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 production of acid from carbohydrates, utilization of organic acids and hydrolysis and decomposition of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, aesculin, urea and hippurate, were evaluated by using the media of Gordon et al. (1974). Allantoin hydrolysis was evaluated in the basal medium suggested by 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 172 agar (Cote et al., 1984).

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

Phylogenetic analysis. The 16S rDNA sequences obtained in this study were aligned manually with actinomycete reference sequences obtained from the Ribosomal Database Project (Maidak et al., 1994) and GenBank in the ARB software environment for sequence data developed by Wolfgang Ludwig and Oliver Strunk (Lehrstuhl für Mikrobiologie, University of Munich, Germany). The program PHYLO_WIN (Galtier et al., 1996) was used to calculate evolutionary distances by the method of Kimura (1980) and linkages by the neighbour-joining method of Saitou & Nei (1987) and to perform maximum-parsimony and maximum-likelihood analyses. The topographies of the trees resulting from neighbour-joining and maximum-parsimony analyses were evaluated by bootstrap analysis of the data with 500 resamplings.

RESULTS AND DISCUSSION

Strain NRRL B-16238T grows well on all of the media evaluated and the gross morphological characteristics on various standard media are shown in Table 1. Soluble pigments were not produced on any of the media tested. Observation of the strain by SEM, in addition to demonstrating the fragmentation of substrate mycelium into rod-shaped elements, showed the presence of sclerotia or pseudosporangium-like bodies on the colony surface, as had been noted by Takahashi et al. (1986) in their original description of this microorganism (Fig. 1a). Swellings near the tips of mycelia

<table>
<thead>
<tr>
<th>Medium</th>
<th>Colour of substrate mycelium</th>
<th>Colour of aerial mycelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC medium 172</td>
<td>Light-orange to light-brown</td>
<td>White to pale-yellowish-pink</td>
</tr>
<tr>
<td>Czapek’s sucrose agar</td>
<td>Yellowish-white</td>
<td>White</td>
</tr>
<tr>
<td>Glycerol/asparagine agar (ISP-5)</td>
<td>Pale-yellow to medium-yellow</td>
<td>Yellowish-white</td>
</tr>
<tr>
<td>Inorganic salts/starch agar (ISP-4)</td>
<td>Light-yellow to brilliant-yellow</td>
<td>White</td>
</tr>
<tr>
<td>Yeast extract/malt extract agar (ISP-2)</td>
<td>Light-yellow to light-brown</td>
<td>White</td>
</tr>
</tbody>
</table>

Table 1. Gross morphological characteristics of Crossiella cryophila NRRL B-16238T

Soluble pigments were not produced on any of the media listed.
The chemotaxonomic profile of NRRL B-16238<sup>T</sup> compared with related taxa is shown in Table 2 and the fatty acid profile is shown in Table 3. The physiological characteristics of NRRL B-16238<sup>T</sup> are listed in the formal description of Crossiella cryophila gen. nov., comb. nov., given below. The phylogenetic position of the proposed genus Crossiella relative to the taxon within or related to the families Actinosynemmataceae and Pseudonocardiaceae can be seen in the nearest-neighbour radial tree shown in Fig. 2.

In their original description of “Nocardiopsis mutabilis subsp. cryophilis”, Takahashi et al. (1986) noted that their isolate had novel morphological characteristics but, based on the chemotaxonomic data, felt that it was best to describe the strain as a subspecies of Nocardiopsis mutabilis. This strain was subsequently transferred to the genus Saccharothrix as Saccharothrix cryophilis as part of a study in which Nocardiopsis mutabilis was transferred to the genus Saccharothrix (Labea & Lechevalier, 1989). DNA-relatedness comparisons made at that time demonstrated conclusively that this strain was not a subspecies of Saccharothrix mutabilis because it only showed 9% DNA relatedness to the type strain of this species. It was noted that Saccharothrix cryophilis exhibited extremely low DNA relatedness to all of the other species of Saccharothrix tested, but there were insufficient additional data available at that time to create a new genus with any confidence.

The recent phylogenetic study of Saccharothrix and related taxa (Labea et al., 2001) revealed that many of the described species of Saccharothrix belong to other genera. Most of the species were contained within the family Actinosynemmataceae, in the genus Lintea (Lintea waywandyensis) or in the newly proposed genus Lechevalieria (Lechevalieria acerocoligens and Lechevalieria flavus), but Saccharothrix cryophilis formed a monophyletic lineage with the genera Actinoalloteichus, Kutzneria and Streptoalloteichus, which all fell phylogenetically between the Actinosynemmataceae and the Pseudonocardiaceae. Its nearest phylogenetic neighbour is the genus Streptoalloteichus, from which it can be distinguished chemotaxonomically and morphologically. Actinoalloteichus forms long spore chains, while Kutzneria and Streptoalloteichus form true sporangia. Streptoalloteichus has also been reported to produce motile spores. The morphological characteristics observed in Saccharothrix cryophilis NRRL B-16238<sup>T</sup>, production of sclerotia or pseudosporangia on the substrate mycelium, are quite different. On the basis of phylogenetic position, chemotaxonomy and novel micromorphology (pseudosporangium production), a new genus is proposed for this strain, to be named Crossiella gen. nov. in recognition of the contributions of Thomas Cross, formerly at the University of Bradford, UK, to actinomycete biology and systematics. The type species of Crossiella is Crossiella cryophila gen. nov., comb. nov. This genus is clearly within the suborder Pseudo-
**Pseudonocardiae**

Actinopolyspora halophila ATCC 27976T/AY5287

Saccharopolyspora

**Saccharomonosporaceae**

Amycolatopsis

Kibdelosporangium aridum ATCC 39325¹ / X53191

**Actinosynnemataceae**

Actinosynnema

Pseudonocardia alni VKM Ac-901T, X76954

Pseudonocardia autotrophica DSM 43210, X76959; Pseudonocardia halophobica DSM 43089T, Z14111; Pseudonocardia hydrocarbonoxydans DSM 43281, X76955; Pseudonocardia nitrificans IFAM 379T, X55609; Pseudonocardia petroleophila ATCC 15777T (= DSM 43193T), X80596; Pseudonocardia saturnea DSM 43195, X76956; Pseudonocardia thermophila ATCC 19285T, X67955; ‘Saccharomonospora caesia’ INMI 19125, X76960; Saccharopolyspora gregorii NCMB 12823T, X76962; Saccharopolyspora hirsuta ATCC 27875T, X53196; Saccharopolyspora hordei ATCC 49856T, X53197; Saccharopolyspora rectivirgula ATCC 33515T, X53194; Saccharothrix austriallensis NRRL 11239T, X114803; Saccharothrix coeruleofusca NRRL B-16115T, X114807; Saccharothrix espanaensis NRRL 15764T, X114807; Saccharothrix longispora NRRL B-16116T, X114809; Saccharothrix mutabilis subsp. capreolus DSM 40225T, X76965; Saccharothrix mutabilis subsp. mutabilis DSM 43853T, X76966; Saccharothrix syringae NRRL B-16468T, X114812; Saccharothrix texensis NRRL B-16134T, X114814. Bar, 0.1 nucleotide substitutions per site.

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**Fig. 2.** Radial phylogenetic tree of the suborder *Pseudonocardineae* calculated from 16 rDNA sequences using Kimura’s evolutionary distance method (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987). This tree shows the relationship of *Crossiella cryophila* NRRL B-16238T to the genera *Actinoalloteichus*, *Kutzneria* and *Streptoalloteichus*. The taxa and sequences included in the genus groups are: *Actinokineospora diospyrosa* NRRL B-24047T, AF114797; *Actinokineospora globicatena* NRRL B-24048T, AF114798; *Actinokineospora inagensis* NRRL B-24050T, AF114799; *Actinokineospora riparia* NRRL B-16432T, AF114802; *Corynebacterium maris* DSM 43850T / X70429; *Kutzneria viridogrisea* NRRL B-16060T, AF114801; *Kutzneria cyaneogenes* IFPO 14455 / AF114801; *Streptoalloteichus indica* DSM 43089T / X55609; *Streptoalloteichus hindustanensis* IFPO 15115 / AB606178. Bar, 0.1 nucleotide substitutions per site. **Pseudonocardinae** Stackebrandt *et al.* 1997, but it cannot be placed clearly within either of the families *Actinomycetaceae* or *Pseudonocardiae* at this time.

**Description of Crossiella gen. nov.**

*Crossiella* (Cross.i.el’a. L. dim. ending -ella; N.L. fem. n. *Crossiella* named for Thomas Cross, a microbiologist at the University of Bradford, who made many contributions to actinomycete biology and systematics).

Aerobic. Gram-positive, non-acid-fast, non-motile actinomycetes. Branched substrate mycelium (approx. 0.5 µm in diameter) and, on some media, aerial mycelia are produced. Vegetative mycelium may fragment into rod-shaped elements and sclerotium-like pseudosporangia may be produced on the substrate mycelium. Swellings may be produced at or near the tip of aerial hyphae. Nocardimycolic acids are absent. Catalase-positive. Contain meso-diaminopimelic acid as the diamino acid and acetylated peptidoglycan. The whole-cell sugar pattern consists of galactose, mannose, rhamnose and ribose. The phospholipid pattern consists of phosphatidyl ethanolamine, phosphatidyl methylethanolamine, phosphatidyl inositol and phosphatidyl inositol mannosides. The predominant menaquinone is MK-9(H₄). Members have a fatty-acid profile rich in branched-chain and saturated components. Phylogenetically, the nearest neighbour is the genus *Streptoalloteichus*. The type species is *Crossiella cryophila*. 

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Description of Crossiella cryophila comb. nov.

Crossiella cryophila (cry.o.phil’a. N.L. adj. cryophila cold-loving, referring to the low permissive temperature range for growth).


This description is based on results from this and earlier studies (Labeda & Lechevalier, 1989). Pale-yellow to light-brown substrate mycelium is produced on most media. White to yellowish-white aerial mycelium is produced, particularly on inorganic salts on most media. White to yellowish substrate mycelium is produced in the presence of 4% NaCl but not in the presence of 5% NaCl. The temperature range for growth is 10–33 °C. The G+C content of the DNA is 71.4 mol% (thermal denaturation midpoint method). Isolated from soil from Shosenkyo, Yamanashi Prefecture, Japan. Produces the antibiotic dopisamine. The type strain of C. cryophila is NRRL B-16238T (= ATCC 51143T = DSM 44230T = IFO 14475T = Y. Okami TS-1980T).

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REFERENCES


