Saccharothrix algeriensis sp. nov., isolated from Saharan soil

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The taxonomic position of a soil isolate, strain SA 233^T, recovered from Saharan soil from Algeria was established using a polyphasic approach. This isolate has been previously reported to produce three novel dithiolopyrrolone antibiotics, and preliminary chemotaxonomic and morphological characteristics suggested that it was representative of a member of the genus *Saccharothrix*. Phylogenetic analysis of the strain from 16S rDNA sequences, along with a detailed analysis of morphological, chemotaxonomic and physiological characteristics, indicates that it belongs to the genus *Saccharothrix* and represents a novel species that is readily distinguished from all recognized *Saccharothrix* species. The name *Saccharothrix algeriensis* sp. nov. is proposed for the isolate, with type strain SA 233^T (=NRRL B-24137^T = DSM 44581^T).

The genus *Saccharothrix*, which currently comprises nine species, is characterized by type III cell wall (*meso*-diaminopimelic acid without glycine), rhamnose, galactose and trace mannose as diagnostic whole-cell sugars, a type PII or PIV phospholipid pattern (phosphatidyl ethanolamine and phosphatidyl hydroxyethanolamine without or with glucosamine-containing phospholipids), presence of MK-9 (II₄) as the predominant menaquinone and absence of mycolic acids (Labeda & Kroppenstedt, 2000). The genera *Lentzea* and *Lechevalieria* differ from *Saccharothrix* by the absence of rhamnose and phosphatidyl hydroxyethanolamine or only of rhamnose, respectively, and constitute a lineage phylogenetically distinct from *Saccharothrix* (Labeda *et al.*, 2001). During studies of the taxonomy and antibiotic production of actinomycetes of Saharan soils from Algeria, we obtained an isolate from soil collected in the palm grove of Adrar oasis, which we have reported to produce three novel dithiolopyrrolone antibiotics (Lamari *et al.*, 2002). Here we describe the characterization and

classification of this isolate, which is proposed as the novel species *Saccharothrix algeriensis* sp. nov. Isolate SA 233^T has been deposited in the Agricultural Research Culture Collection and in the Deutsche Sammlung von Mikroorganismen und Zellkulturen under numbers NRRL B-24137^T and DSM 44581^T, respectively.

Strain SA 233^T was isolated from a Saharan soil sample collected at a palm grove in Adrar, Algeria, by a dilution agar plating method using humic acid/B vitamin agar medium (Hayakawa & Nonomura, 1987) supplemented with streptomycin sulphate (10 µg ml⁻¹) and actidione (50 µg ml⁻¹). Biomass for chemotaxonomic analysis was grown on yeast extract/malt extract broth, on a rotary shaker for 4 days at 30 °C, harvested by centrifugation and washed twice with distilled water.

Detailed observation of mycelium morphology was performed using a scanning electron microscope (Stereoscan 260; Cambridge Instruments). Cultural characteristics observed on media from the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966), nutrient agar and Bennett agar (Waksman, 1961) were recorded after 7–14 days incubation at 28 °C. Colours were determined according to the ISCC–NBS centroid colour chart (Kelly & Judd, 1976).

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Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SA 233^T is AY054972.

Tryptone/yeast extract agar (ISP medium 1), peptone/yeast extract/iron agar (ISP medium 6) and tyrosine agar (ISP medium 7) (Shirling & Gottlieb, 1966) were used to determine melanoid pigment production. Decomposition of adenine, guanine, hypoxanthine, tyrosine and xanthine were determined as described by Gordon *et al.* (1974), and arbutin and aesculin decomposition, gelatin liquefaction, starch hydrolysis and nitrate reductase production were determined as described by Marchal & Bourdon (1973). Utilization of Tween 80 and testosterone was determined by the methods of Sierra (1957) and Goodfellow (1971), respectively. Carbohydrate and organic acid assimilation were determined as described by Goodfellow (1971) and Gordon *et al.* (1974), respectively. Sensitivity to lysozyme was studied using the method of Gordon & Barnett (1977). Sensitivity to phenol, potassium tellurite, sodium azide, sodium chloride and crystal violet were determined on glucose/yeast extract agar as described by Athalye *et al.* (1985). Growth at different temperatures and pH and in the presence of various antibiotics was determined on the same media.

The isomeric form of diaminopimelic acid and predominant whole-cell sugars were detected following standard procedures described by Becker *et al.* (1964) and Lechevalier & Lechevalier (1970). Phospholipids and mycolic acids were analysed using the procedure of Minnikin *et al.* (1977, 1980). The fatty acid profile was determined by the method of Grund & Kroppenstedt (1989).

Genomic DNA for sequencing was isolated, purified and sequenced following the procedures described by Labeda & Kroppenstedt (2000). The 16S rDNA sequence was manually aligned 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 W. Ludwig and O. Strunk (Lehrstuhl für Mikrobiologie, University of Munich, Germany). Evolutionary distances were calculated within ARB by the method of Kimura (1980) and linkages by the neighbour-joining method of Saitou & Nei (1987); maximum-parsimony and maximum-likelihood analyses were also performed in ARB. The topologies of the trees resulting from neighbour-joining and maximum-parsimony analyses were evaluated by bootstrap analysis of the data with 500 resamplings.

Genomic DNA was isolated as described by Labeda (1998) and DNA–DNA relatedness between strains was determined spectrophotometrically in 5 × SSC (1 × SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) and 20% dimethyl sulphoxide at 66 °C (melting point – 23 °C) by the method of De Ley *et al.* (1970).

Strain SA 233^T exhibited good growth on ISP-2, ISP-5 and Bennett agar, with well-developed, yellow orange aerial mycelium that fragmented into rod-shaped spores. The spores had a smooth surface (Fig. 1) and were non-motile. No endospores, sporangia, sclerotia or synnemata were

Fig. 1. Scanning electron micrograph of spore chains of strain SA 233^T grown on yeast extract/malt extract agar (ISP medium 2) for 10 days at 30 °C. Bar, 5 µm.

observed. Growth was moderate on ISP-3 and nutrient agar and poor on ISP-4 agar, with poorly developed aerial mycelium. The substrate mycelium exhibited little or no fragmentation on either solid or liquid media. The substrate mycelium was vivid yellow, orange yellow or yellow brown. A bright yellow soluble pigment was produced on ISP-2 and ISP-3 agar, whereas a yellow brown soluble pigment was produced on Bennett agar. The isolate did not produce melanoid pigments on ISP-1, ISP-6 or ISP-7 agar.

Cell-wall hydrolysates contained *meso*-diaminopimelic acid but not glycine and whole-cell hydrolysates contained galactose, mannose and rhamnose, in addition to glucose and ribose, typical of cell wall type III (Lechevalier & Lechevalier,

Table 1. Fatty acid profile of *Saccharothrix algeriensis* sp. nov. SA 233^T

Fatty acid identities were determined from the Microbial Identification System software (MIDI Inc.) peak-naming table.

Fatty acid	Percentage of total
iso-C _{14:0}	3.46
iso-C _{15:0}	10.06
anteiso-C _{15:0}	1.80
C _{15:1} B	2.11
iso-C _{16:1} H	14.00
iso-C _{16:0}	31.26
<i>cis</i> -9-C _{16:1}	3.92
9?-Methyl-C _{16:0}	3.42
iso-C _{17:0}	1.65
anteiso-C _{17:0}	3.00
<i>cis</i> -9-C _{17:1}	7.37
iso-2-hydroxy-C _{16:0}	10.44
10-Methyl-C _{17:0}	1.13

1970). Diagnostic phospholipids detected were phosphatidyl ethanolamine (with phosphatidyl hydroxy-ethanolamine) and glucosamine-containing phospholipids, corresponding to phospholipids type PIV (Lechevalier *et al.*, 1977). Mycolic acids were not detected. The predominant fatty acid was iso-branched hexadecanoate (iso-C_{16:0}), and significant amounts of iso-C_{16:1} (position of the unsaturation unknown), iso-2-hydroxy-C_{16:0} and iso-C_{15:0} fatty acids were also present. The cellular fatty acid composition of the strain SA 233^T is given in Table 1. The morphological and chemical characteristics described above clearly support the placement of strain SA 233^T within the genus *Saccharothrix*.

Strain SA 233^T is physiologically different from other recognized *Saccharothrix* species, as can be seen from the differential physiological characteristics given in Table 2. In addition, a comparison with the physiologically nearest species, *Saccharothrix australiensis* NRRL 11239^T, showed

differences in characteristics of the cultures (colour of aerial and substrate mycelia and colour of diffusible pigments) and in the utilization of cellobiose, dextrin, mannose, sorbitol, erythritol, sodium acetate, sodium citrate and sodium propionate. Complete physiological characteristics of strain SA 233^T are given in the formal description below.

The phylogenetic relationship between strain SA 233^T and the other recognized species of *Saccharothrix* is seen in the neighbour-joining dendrogram in Fig. 2. Maximum-parsimony calculation resulted in a similar tree topology. The similarity of the 16S rDNA sequence of strain SA 233^T to those of the other species of the genus *Saccharothrix* ranged from 97.5 to 98.8 %, with *Saccharothrix australiensis* NRRL 11239^T having the closest match. Other species within the genus *Saccharothrix* that have been determined to be distinct based on DNA–DNA relatedness have 16S rRNA gene sequence similarities in this range of values.

Table 2. Differential properties of *Saccharothrix algeriensis* sp. nov. compared with recognized species of the genus *Saccharothrix*

Strains: 1, *S. algeriensis* sp. nov. SA 233^T; 2, *S. australiensis* NRRL 11239^T; 3, *S. coeruleofuscus* DSM 43679^T; 4, *S. coeruleoviolacea* DSM 43935^T; 5, *S. espanaensis* NRRL 15764^T; 6, *S. longispora* DSM 43749^T; 7, *S. mutabilis* subsp. *mutabilis* NRRL B-16077^T; 8, *S. mutabilis* subsp. *capreolus* DSM 40225^T; 9, *S. syringae* DSM 43886^T; 10, *S. tangerinus* JCM 10302^T; 11, *S. texasensis* NRRL B-16134^T. Data for reference species were taken from Labeda (2002). –, Negative reaction; +, positive reaction; w, weak positive reaction.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Decomposition of:											
Adenine	–	–	–	–	–	+	–	–	–	–	–
Hypoxanthine	–	–	–	–	+	–	+	+	–	+	+
Starch	–	–	+	–	–	+	+	+	+	+	+
Tyrosine	+	+	–	–	–	+	+	+	+	+	+
Production of:											
Soluble pigments	+	+	–	+	–	–	–	–	+	–	–
Nitrate reductase	+	+	–	+	w	+	+	–	–	–	+
Assimilation of citrate	+	–	–	+	w	+	+	–	–	+	–
Acid from:											
Arabinose	–	–	+	+	–	+	+	+	+	+	+
Dextrin	–	+	+	+	–	+	+	+	+	+	+
Inositol	–	–	–	–	–	–	+	+	–	+	+
Lactose	–	–	+	+	–	+	+	–	+	+	+
Melibiose	–	–	–	+	–	–	+	+	+	+	+
Raffinose	–	–	+	–	–	–	+	–	+	+	–
Rhamnose	–	–	+	+	–	+	–	–	+	+	+
Sorbitol	–	+	+	–	–	–	–	–	–	+	–
Sucrose	–	–	+	–	+	+	+	–	+	+	+
Xylose	–	–	+	+	w	+	+	+	+	+	+
Methyl α -D-glucoside	–	–	+	w	–	–	+	–	–	w	+
Growth in the presence of:											
4 % NaCl	+	+	+	–	+	+	–	+	+	+	–
5 % NaCl	–	–	+	–	–	+	–	+	+	w	–
Growth at:											
37 °C	+	+	+	+	+	+	+	+	+	–	+
45 °C	+	+	+	+	–	–	+	+	+	–	–

Fig. 2. Phylogenetic tree for species of the genus *Saccharothrix* calculated from almost complete 16S rDNA sequences using Kimura's evolutionary distance methods (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987). This illustrates the taxonomic position of *Saccharothrix algeriensis* sp. nov. NRRL B-24137^T (=SA 223^T) relative to the other species of the genus. Bar, 0.01 nucleotide substitutions per site.

Strain SA 233^T was determined to have only 55.9 % genomic DNA–DNA relatedness (based on a mean of duplicate determinations) to *S. australiensis* NRRL 11239^T, the phylogenetically closest species of the genus *Saccharothrix*, which is clearly below the 70 % relatedness guideline proposed by Wayne *et al.* (1987) for the delineation of separate species. All of the data support the designation of strain SA 233^T as representing a novel species of the genus *Saccharothrix*, for which we propose the name *Saccharothrix algeriensis* sp. nov.

Description of *Saccharothrix algeriensis* sp. nov.

Saccharothrix algeriensis (al.ger.i.en'sis. N.L. fem. adj. *algeriensis* of Algeria, where the type strain originated).

Aerobic, Gram-positive, catalase-positive. Forms a copious yellow orange aerial mycelium that fragments into rod-shaped spores. Spore surface is smooth. Substrate mycelium is vivid yellow, orange yellow or yellowish brown. A bright yellow soluble pigment is produced but no melanoid pigment. Growth occurs between 18 and 45 °C but not at 48 °C. Utilizes D-fructose, D-galactose, D-glucose, maltose, D-trehalose, glycerol, acetate, citrate, pyruvate and succinate as carbon sources, but not L-arabinose, D-cellobiose, dextrin, D-lactose, D-mannose, D-melezitose, melibiose, methyl α -D-glucoside, D-raffinose, L-rhamnose, D-ribose, sucrose, D-xylose, adonitol, dulcitol, *meso*-erythritol, *meso*-inositol, D-mannitol, D-sorbitol, benzoate, butyrate, oxalate, propionate and tartrate. Aesculin, casein, gelatin, Tween 80 and tyrosine are hydrolysed. No hydrolysis of adenine, arbutin, guanine, hypoxanthine, starch, testosterone or xanthine. Nitrate reductase is produced. Growth occurs at pH 5 and 9 and in the presence of 0.005 % lysozyme, 0.01 % potassium tellurite, 0.001 % crystal violet and 0.05 % phenol. Growth does not occur in the presence of 5 % NaCl, 0.001 % sodium azide and 0.1 % phenol. Susceptible to chloramphenicol (25 μ g ml⁻¹), erythromycin (10 μ g ml⁻¹), kanamycin (25 μ g ml⁻¹), novobiocin (10 μ g ml⁻¹) and penicillin (10 μ g ml⁻¹) but resistant to cycloserin (10 μ g ml⁻¹), gentamicin (5 μ g ml⁻¹), oxytetracycline (25 μ g ml⁻¹), rifampicin (5 μ g ml⁻¹), streptomycin (10 μ g ml⁻¹) and vancomycin (5 μ g ml⁻¹). Type III cell wall (*meso*-diaminopimelic acid, galactose, mannose and rhamnose in whole-cell hydrolysates). Phospholipids

type PIV (phosphatidyl ethanolamine and glucosamine-containing phospholipids). The predominant fatty acid is iso-C_{16:0} (31.26 %), followed by iso-H-C_{16:0} (14.00 %), iso-2-hydroxy-C_{16:0} (10.44 %) and iso-C_{15:0} (10.06 %). Mycolic acids are absent.

The type strain is SA 233^T (=NRRL B-24137^T=DSM 44581^T), isolated from a Saharan soil sample collected at a palm grove in Adrar, Algeria. The species description is based on a single strain and hence serves as the strain description.

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References

- Athalye, M., Goodfellow, M., Lacey, J. & White, R. P. (1985). Numerical classification of *Actinomyces* and *Nocardia*. *Int J Syst Bacteriol* 35, 86–98.
- Becker, B., Lechevalier, M. P., Gordon, R. E. & Lechevalier, H. A. (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl Microbiol* 12, 421–423.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* 12, 133–142.
- Goodfellow, M. (1971). Numerical taxonomy of some nocardioform bacteria. *J Gen Microbiol* 69, 33–80.
- Gordon, R. E. & Barnett, D. A. (1977). Resistance to rifampin and lysozyme of strains of some species of *Mycobacterium* and *Nocardia* as a taxonomic tool. *Int J Syst Bacteriol* 27, 176–178.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C.-H. N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* 24, 54–63.
- Grund, E. & Kroppenstedt, R. M. (1989). Transfer of five *Nocardia* species to the genus *Saccharothrix* Labeda *et al.*, 1984. *Syst Appl Microbiol* 12, 267–274.

- Hayakawa, M. & Nonomura, H. (1987).** Humic acid–vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.
- Kelly, K. L. & Judd, D. B. (1976).** *Color. Universal Language and Dictionary of Names* (National Bureau of Standards Special Publication 440). Washington, DC: US Department of Commerce.
- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Labeda, D. P. (1998).** DNA relatedness among the *Streptomyces fulvissimus* and *Streptomyces griseoviridis* phenotypic cluster groups. *Int J Syst Bacteriol* **48**, 829–832.
- Labeda, D. P. (2002).** The family Actinosynnemataceae. In *The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community*, 3rd edn, release 3.9, 1 April 2002. Edited by M. Dworkin *et al.* New York: Springer. <http://www.prokaryotes.com>
- Labeda, D. P. & Kroppenstedt, R. M. (2000).** Phylogenetic analysis of *Saccharothrix* and related taxa: proposal for Actinosynnemataceae fam. nov. *Int J Syst Evol Microbiol* **50**, 331–336.
- Labeda, D. P., Hatano, K., Kroppenstedt, R. M. & Tamura, T. (2001).** Revival of the genus *Lentzea* and proposal for *Lechevalieria* gen. nov. *Int J Syst Evol Microbiol* **51**, 1045–1050.
- Lamari, L., Zitouni, A., Boudjella, H., Badji, B., Sabaou, N., Lebrihi, A., Lefebvre, G., Seguin, E. & Tillequin, F. (2002).** New dithiopyrrolone antibiotics from *Saccharothrix* sp. SA 233. I. Taxonomy, fermentation, isolation, and biological activities. *J Antibiot* **55**, 696–701.
- Lechevalier, M. P. & Lechevalier, H. A. (1970).** Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **34**, 435–444.
- Lechevalier, M. P., de Bièvre, C. & Lechevalier, H. A. (1977).** Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Maidak, B. L., Larsen, N., McCaughey, M. J., Overbeek, R., Olsen, G. J., Fogel, K., Blandy, J. & Woese, C. R. (1994).** The Ribosomal Database Project. *Nucleic Acids Res* **22**, 3485–3487.
- Marchal, N. & Bourdon, J. L. (1973).** *Milieux de Culture et Identification Biochimique des Bactéries*. Paris: Doin (in French).
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977).** Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–107.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980).** Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr* **188**, 221–233.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Sierra, G. (1957).** A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of contact between the cells and fatty substrates. *Antonie van Leeuwenhoek* **23**, 15–22.
- Waksman, S. A. (1961).** Classification, identification, and descriptions of genera and species. In *The Actinomycetes*, vol. 2, pp. 331–332. Baltimore: Williams & Wilkins.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987).** Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.