Reclassification of *Amycolatopsis rugosa* Lechevalier *et al.* 1986 as *Prauserella rugosa* gen. nov., comb. nov.

Seung Bum Kim and Michael Goodfellow

Author for correspondence: Seung B. Kim. Tel: +44 191 222 5922. Fax: +44 191 222 5228.
e-mail: s.b.kim@ncl.ac.uk

Department of
Agricultural and
Environmental Science,
University of Newcastle,
Newcastle upon Tyne
NE1 7RU, UK

An almost complete sequence of the 16S rDNA of the type strain of *Amycolatopsis rugosa* was determined following direct sequencing of the amplified gene. The sequence was aligned with those of representatives of the family *Pseudonocardiaceae* and related actinomycetes and phylogenetic trees were inferred by using three tree-making algorithms. The organism formed a distinct clade within the evolutionary radiation occupied by the family *Pseudonocardiaceae*. It was also readily distinguished from all of the validly described genera classified in this taxon by using a combination of chemical and morphological markers. On the basis of these genotypic and phenotypic differences, the name *Prauserella* gen. nov. is proposed for a new genus containing the previously misclassified *Amycolatopsis rugosa*. The type strain of *Prauserella rugosa* is DSM 43194^T (= ATCC 43014^T = NCIMB 8926^T).

Keywords: *Prauserella rugosa*, 16S rDNA sequencing, polyphasic taxonomy

INTRODUCTION

Nocardia rugosa was proposed by Di Marco & Spalla (1957) to accommodate actinomycetes isolated from cattle rumen. The taxon was well described but a type strain was not designated until the species was transferred to the genus *Amycolatopsis* as *Amycolatopsis rugosa* (Lechevalier *et al.*, 1986). The type strain forms a substrate mycelium which has the following characteristics: it undergoes fragmentation (Di Marco & Spalla, 1957; Lechevalier *et al.*, 1986); it contains meso-diaminopimelic acid, arabinose and galactose (Lechevalier *et al.*, 1986; Whitehead, 1989), that is, it has a wall chemotype IV *sensu* Lechevalier & Lechevalier (1970); it contains complex mixtures of branched-chain and saturated fatty acids (Henssen *et al.*, 1987; Mertz & Yao, 1993); it lacks mycolic acids (Lechevalier *et al.*, 1986; Henssen *et al.*, 1987) and contains muramic acid in the *N*-acetylated form (Henssen *et al.*, 1987); it has di- and tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue (Lechevalier *et al.*, 1986; Yassin *et al.*, 1991) and has major amounts of diphosphatidylglycerol, phosphatidylglycerol, phos-

phatidylinositol, phosphatidylethanolamine and phosphatidylmethylethanolamine, with the latter two components acetylated to hydroxy fatty acids (Lechevalier *et al.*, 1986; Yassin *et al.*, 1993). The G + C content of the DNA of the type strain has been reported to be 67.0 (Akimov *et al.*, 1989) and 68.9 mol % (Lechevalier *et al.*, 1986).

It seems likely that *A. rugosa* is misclassified in the genus *Amycolatopsis*. Representatives of the taxon have been repeatedly assigned to a homogeneous cluster, sharply separated from clusters corresponding to validly described species of the genera *Amycolatopsis*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora* and *Saccharopolyspora*, in extensive numerical phenetic surveys of wall-chemotype IV actinomycetes lacking mycolic acids (Mattinson-Rose, 1986; Whitehead, 1989). The type strain of *A. rugosa* was also separated from representatives of most other *Amycolatopsis* species in studies of DNA–DNA relatedness (Labeda, 1995), cellular fatty acids (Mertz & Yao, 1993) and phage host-range (Lechevalier *et al.*, 1986) and in non-numerically based phenotypic studies (De Boer *et al.*, 1990).

Sequencing of 16S rDNA provides a powerful way of distinguishing between actinomycetes that have proved difficult to classify by standard phenotypic methods (Chun & Goodfellow, 1995; Chun *et al.*,

The GenBank accession number for the 16S rDNA sequence of strain DSM 43194^T is AF051342.

1997; Kim *et al.*, 1998). The present study was designed to determine whether 16S rDNA sequencing could be used to help resolve the taxonomic status of *A. rugosa*.

METHODS

Organisms and culture conditions. The type strain of *A. rugosa* was received from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) and from the National Collection of Industrial and Marine Bacteria (NCIMB; Aberdeen, UK). Cultures of strains DSM 43194^T and NCIMB 8926^T were maintained on modified Bennett's agar plates (Jones, 1949) and as suspensions in 20% (v/v) glycerol at -20 °C.

Morphology. The colonial and pigmentational properties of the test strains were examined on plates of inorganic salts-starch agar (ISP medium 4; Difco) (Shirling & Gottlieb, 1966), nutrient agar (Difco) and Czapek Dox-yeast extract agar (Cross & Attwell, 1974) after 14 d at 28 °C.

16S rDNA sequencing. Extraction of chromosomal DNA, PCR amplification and direct sequencing of the amplified products from the test strains were performed according to the procedure described by Kim *et al.* (1996). A primer designated 342r (5' GGAGTCTGGGCCGTGTCTC 3') was used for direct sequencing in addition to the previously described primers (Chun & Goodfellow, 1995).

Phylogenetic analysis. The 16S rDNA sequences were aligned manually with the AL16S program (Chun, 1995) against corresponding nucleotide sequences of representatives of the family *Pseudonocardiaceae* (Stackebrandt *et al.*, 1997) retrieved from the EMBL and GenBank databases. Phylogenetic trees were inferred by using the Fitch-Margoliash (Fitch & Margoliash, 1967), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) treeing algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the Fitch-Margoliash and neighbour-joining methods were generated as described by Jukes & Cantor (1969). Tree topologies were evaluated by bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the SEQBOOT and CONSENSE options from the PHYLIP suite of programs (Felsenstein, 1993). The root position of the tree based on the neighbour-joining method was estimated by using four outgroup organisms, *Arthrobacter globiformis* (GenBank accession no. M23411), *Bacillus subtilis* (K00637), *Escherichia coli* (J01695) and *Streptomyces ambofaciens* (M27245), as described by Swofford & Olsen (1990).

RESULTS AND DISCUSSION

The almost complete 16S rDNA sequences (1519 nucleotides) obtained for *A. rugosa* strains DSM 43194^T and NCIMB 8926^T were identical. Comparison of this common sequence with those of corresponding sequences of representatives of the family *Pseudonocardiaceae* (Warwick *et al.*, 1994; Stackebrandt *et al.*, 1997) showed the organism to be loosely associated with the genus *Saccharomonospora* in the phylogenetic trees based on the neighbour-joining and Fitch-Margoliash algorithms (Fig. 1). In the corresponding maximum-likelihood tree, the strain formed a distinct clade within the evolutionary radiation encompassed by the family *Pseudonocardiaceae*. The test strain also

showed a pattern of 16S rDNA sequences characteristic of this family (Stackebrandt *et al.*, 1997). The family *Pseudonocardiaceae* recognized by Stackebrandt *et al.* (1997) included the genera *Actinosynnema*, *Saccharothrix* and *Streptoalloteichus*; these taxa were omitted from the family by Warwick *et al.* (1994), who considered that they might form a sister group to the family *Pseudonocardiaceae*. This now seems to be the case, as Labeda (1998) has suggested that the genera *Actinokineospora*, *Actinosynnema*, *Lentzea* and *Saccharothrix* should be assigned to a putative new taxon, the family 'Actinosynnemaceae'. The 16S rDNA data suggest that the genera *Kutzneria* and *Streptoalloteichus* might also belong to this group (Fig. 1). These propositions leave the family *Pseudonocardiaceae* as a relatively homogeneous group (Embley *et al.*, 1988; Warwick *et al.*, 1994).

The chemical and morphological properties of *A. rugosa* DSM 43194^T show that it is correctly classified in the family *Pseudonocardiaceae sensu stricto*. Thus, the organism forms a substrate mycelium that fragments into irregularly shaped elements (Di Marco & Spalla, 1957; Lechevalier *et al.*, 1986), produces an acetylated peptidoglycan (Henssen *et al.*, 1987), has wall chemotype IV (Lechevalier *et al.*, 1986; Whitehead, 1989), is rich in branched-chain and saturated fatty acids (Henssen *et al.*, 1987; Mertz & Yao, 1993), contains di- and tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue (Lechevalier *et al.*, 1986; Yassin *et al.*, 1991), contains diphosphatidylglycerol and phosphatidylethanolamine as diagnostic polar lipids (Lechevalier *et al.*, 1986; Yassin *et al.*, 1993) and has DNA with a high G + C content (Lechevalier *et al.*, 1986; Akimov *et al.*, 1989) but which lacks mycolic acids (Lechevalier *et al.*, 1986; Henssen *et al.*, 1987).

Two main types of phospholipid patterns have been detected in members of the family *Pseudonocardiaceae* (Embley *et al.*, 1988; Lechevalier *et al.*, 1977, 1986; Shearer *et al.*, 1986). Representatives of the genera *Pseudonocardia* and *Saccharopolyspora* contain phosphatidylcholine and phosphatidylethanolamine derivatives as their major phospholipids (phospholipid type III *sensu* Lechevalier *et al.*, 1977). *A. rugosa*, together with members of the genera *Amycolatopsis*, *Kibdelosporangium* and *Saccharomonospora*, lack phosphatidylcholine and contain phosphatidylethanolamine and derivatives as major phospholipids (phospholipid type II).

The highest mean 16S rDNA identities found between the type strain of *A. rugosa* and other members of the family *Pseudonocardiaceae sensu stricto* were with representatives of the genera *Amycolatopsis* (93.8%), *Pseudonocardia* (92.5%), *Saccharomonospora* (94.7%) and *Thermocrispum* (93.2%). Mean identities within or just outside this range are found between established genera classified within this family: for instance, between the genera *Amycolatopsis* and *Saccharopolyspora* (93.4%) and between *Pseudonocardia* and *Saccharomonospora* (92.1%). *A. rugosa* can also be

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987), based on 16S rDNA sequences, showing relationships between *A. rugosa* strain DSM 43194^T and representatives of the family *Pseudonocardiaceae* and related taxa. Asterisks indicate branches recovered with all three methods; f and m indicate branches that were also recovered by using the Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum-parsimony (Kluge & Farris, 1969) treeing algorithms, respectively. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are given. The arrow indicates the estimated position of the four root organisms. The scale bar indicates 0.01 nucleotide substitutions per nucleotide position.

Table 1. Diagnostic characters that separate *A. rugosa* from representatives of genera classified in the family *Pseudonocardiaceae sensu stricto*

Data were taken from earlier studies: Lechevalier *et al.* (1986); Shearer *et al.* (1986); Labeda (1987); Henssen *et al.* (1987); Akimov *et al.* (1989); Goodfellow *et al.* (1989); Korn-Wendisch *et al.* (1989, 1995); Yoshida *et al.* (1991); Reichert *et al.* (1998). +, Present or positive; –, absent or negative; v, variable; ND, not determined.

Character	<i>A. rugosa</i>	<i>Actinopolyspora</i>	<i>Amycolatopsis</i>	<i>Kibdelosporangium</i>	<i>Pseudonocardia</i>	<i>Saccharomonospora</i>	<i>Saccharopolyspora</i>	<i>Thermocrispum</i>
Morphological features:								
Acropetal budding	–	–	–	–	+	–	–	–
Aerial hyphae	–	+	+	+	+	+	+	+
Spores on aerial hyphae	–	In chains	In chains	In chains	In chains	Singly	In chains	In chains
Fragmentation of substrate hyphae	+	v	+	+	v	–	+	–
Spores on substrate hyphae	–	–	+	–	+	–	v	ND
Sporangia-like structures	–	–	–	+	–	–	–	+
Chemical properties:								
Phospholipid type*	2	3	2	2	2, 3	2	3	2
Diagnostic whole-cell sugars	Arabinose, galactose	Arabinose, galactose	Arabinose, galactose	Arabinose, galactose	Arabinose, galactose	Arabinose, galactose	Arabinose, galactose	None
Predominant menaquinone(s)†	9(H ₂ , H ₄)	9(H ₂ , H ₆), 10(H ₄)	9(H ₂ , H ₄ , H ₆)	9(H ₄)	8(H ₂ , H ₄)	8(H ₄), 9(H ₄)	9(H ₄), 10(H ₄)	9(H ₄)
DNA G+C content (mol %)	67.0–68.9	64–68	66–73	66	69–74	66–70	70–77	69–73
Extreme halophile	–	+	–	–	–	–	–	–

* Abbreviated as 2, phosphatidylethanolamine and 3, phosphatidylcholine (with phosphatidylethanolamine, phosphatidyl-methylethanolamine and phosphatidylglycerol variable; no phospholipids containing glucosamine) (Lechevalier *et al.*, 1977).

† Abbreviations are exemplified by 9(H₄), menaquinones having two of the nine isoprene units hydrogenated.

distinguished from other members of the family *Pseudonocardiaceae sensu* Warwick *et al.* (1994) by using a combination of biochemical, chemical, morphological and physiological markers (Table 1). The

failure of *A. rugosa* strains DSM 43194^T and NCIMB 8926^T to form aerial hyphae on Czapek Dox-yeast extract, inorganic salts-starch and nutrient agars is consistent with observations from previous studies (Di

The genotypic and phenotypic data show that *A. rugosa* DSM 43194^T (=NCIMB 8926^T) does not belong to the genus *Amycolatopsis* but should be classified in a new genus within the family *Pseudonocardiaceae* Warwick *et al.* (1994). It is therefore proposed that *Amycolatopsis rugosa* be classified in the genus *Prauserella* gen. nov. as *Prauserella rugosa* gen. nov., comb. nov.

Description of *Prauserella* gen. nov.

Prauserella (Prau.se.rel'la. M.L. dim. ending -ella; M. L. fem. n. *Prauserella* named after Helmut Prauser, a German microbiologist who made many contributions to actinomycete systematics). The description is based on data taken from this and earlier studies (Di Marco & Spalla, 1957; Lechevalier *et al.*, 1986; Henssen *et al.*, 1987).

Aerobic, Gram-positive, non-acid-alcohol-fast, non-motile actinomycetes that form an extensively branched substrate mycelium (0.6–0.8 µm in diameter) which fragments into irregular rods within 24–48 h on rich media. Aerial hyphae are not formed. Produce cream to yellowish, glistening colonies that may be wrinkled or folded. Good growth occurs on asparagine, modified Bennett's, Czapek Dox-yeast extract, glycerine and nutrient agars. Brownish, soluble pigments are formed on some media. The temperature range for growth is 10–45 °C with the optimum temperature of 34 °C. Grow optimally between pH 6.8 and 7.2. Contain *meso*-diaminopimelic acid as the diamino acid, an acetylated peptidoglycan, major amounts of arabinose and galactose, di- and tetra-hydrogenated menaquinones with nine isoprene units as the predominant isoprenologue and diphosphatidyl-glycerol and phosphatidylethanolamine as diagnostic polar lipids, have a fatty-acid profile rich in branched-chain and saturated components and lack mycolic acids. The genus belongs to the family *Pseudonocardiaceae* Warwick *et al.* (1994). The type and only species is *Prauserella rugosa*.

Description of *Prauserella rugosa* gen. nov., comb. nov.

Prauserella rugosa (ru.go'sa. L. adj. *rugosa* wrinkled). The description is based on results from this and earlier studies (Di Marco & Spalla, 1957; Lechevalier *et al.*, 1986; Akimov *et al.*, 1989; Whitehead, 1989; Mertz & Yao, 1993).

Cream coloured, irregular, flat and veined colonies (10 × 6 mm) formed on Czapek Dox-yeast extract agar. Arbutin, casein, hypoxanthine, L-tyrosine, tributyrin and Tweens 20, 40, 60 and 80 are degraded but adenine, elastin, starch and testosterone are not. Aesculin, allantoin and urea are hydrolysed and phosphatase is produced but nitrate is not reduced. Hydrogen sulphide is not formed but the organism is

sensitive to lysozyme. Acetate, benzoate, lactate, malate, propionate, pyruvate and succinate are decarboxylated but citrate, mucate, oxalate and tartrate are not. Acid is formed from adonitol, arabinose, erythritol, fructose, galactose, glucose, glycerol, mannitol, mannose, rhamnose, salicin, trehalose and xylose but not from cellobiose, dextrin, inositol, lactose, maltose, melibiose, methyl α-D-glucoside, raffinose, sorbitol, sucrose or methyl β-D-xyloside. Utilizes adonitol, amygdalin, (+)-L-arabinose, arbutin, cellobiose, dextrin, erythritol, fructose, galactose, glucose, glycerol, glycogen, lactose, maltose, mannitol, mannose, rhamnose, ribose, salicin, sucrose, trehalose, xylitol and xylose as sole sources of carbon for energy and growth but not arabitol, dulcitol, (+)-D- or (–)-L-fucose, gentibiose, inositol, sorbitol or turanose (all sugars at 1%, w/v). Similarly, L-alanine, androsterone, cholesterol, ergosterol, L-glycine, L-proline, protocatechuic acid, shikimic acid, butyrate, propionate, pyruvate, succinate and L-tyrosine are used as sole carbon sources but not D-α-alanine, L-arginine, L-asparagine, catechol, L-citrulline, creatine, L-cysteine, L-citrulline, ferulic acid, histamine, L-hydroxyproline, phthallic acid, progesterone, quinic acid, L-serine, fumarate, tartrate, syringaldehyde, L-threonine or vanillin (all at 0.1%, w/v). Growth occurs in the presence of cephaloridine hydrochloride (2 µg ml⁻¹), demeclocycline hydrochloride (2 µg ml⁻¹), lincomycin hydrochloride (10 µg ml⁻¹), neomycin sulphate (3 µg ml⁻¹), oleandomycin phosphate (2 µg ml⁻¹), penicillin G (10 µg ml⁻¹), streptomycin sulphate (16 µg ml⁻¹) and vancomycin hydrochloride (0.25 µg ml⁻¹) but not in the presence of cephaloridine hydrochloride (25 µg ml⁻¹), chloramphenicol (25 µg ml⁻¹), gentamicin sulphate (0.5 µg ml⁻¹), kanamycin sulphate (5 µg ml⁻¹), rifampicin (2 µg ml⁻¹), tetracycline hydrochloride (5 µg ml⁻¹) or tobramycin sulphate (8 µg ml⁻¹). Similarly, growth occurs in the presence of bismuth citrate (1 µg ml⁻¹), crystal violet (1 µg ml⁻¹), phenol (100 µg ml⁻¹), phenyl ethanol (0.1%, v/v), potassium tellurite (10 µg ml⁻¹), sodium azide (1 µg ml⁻¹), teepol (100 µg ml⁻¹), tetrazolium (100 µg ml⁻¹), thallous acetate (10 µg ml⁻¹) and sodium chloride (13%, w/v) but not in the presence of adenine (0.4% w/v), crystal violet (10 µg ml⁻¹), phenyl ethanol (0.4%, v/v), potassium tellurite (100 µg ml⁻¹), sodium azide (100 µg ml⁻¹) or thallous acetate (100 µg ml⁻¹). The DNA G + C content of the type strain has been reported to be 67.0/68.9 mol% by the *T_m* method. The type strain produces vitamin B₁₂. Isolated from cattle rumen. The type strain of *Prauserella rugosa* is DSM 43194^T (=ATCC 43014^T = NCIMB 8926^T).

ACKNOWLEDGEMENTS

Part of this work was supported by a BBSRC grant (R185/05688/01). S.B.K. is grateful for support from a Chevening–MOST scholarship. The authors are indebted to R. M. Kroppenstedt for providing the type strain of *Amycolatopsis rugosa* and to K. P. Schaal for help with the nomenclature.

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