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ORIGINAL ARTICLE

Amycolatopsis roodepoortensis sp. nov. and *Amycolatopsis speibonae* sp. nov.: antibiotic-producing actinobacteria isolated from South African soils

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Two novel members of the genus *Amycolatopsis* were isolated from soil samples collected in South Africa. Strains JS72^T and M29^T clustered in the same clade in the 16S-rRNA, *gyrB*-16S-rRNA and *gyrB-recN* gene trees. Both strains showed anti-mycobacterial activity. The *oxyB* P450 monooxygenase B gene required for the production of glycopeptide antibiotics was detected in both strains, while strain JS72^T was also shown to contain the 3-amino-5-hydroxy-benzoic acid synthase gene, which is required for the production of the ansamycin class of antibiotics. Genetic distance values (based on the *gyrB* and *recN* genes) were calculated between strains JS72^T and M29^T and their closest phylogenetic relatives. The values for strain JS72^T were all above the threshold values of 0.02 and 0.04, respectively, that have been proposed to distinguish *Amycolatopsis*-type strains. The *gyrB*-based values for strain M29^T were above the threshold for all but one strain; the *recN*-based values were all above the threshold. These data, along with DNA–DNA hybridization data, showed that strains JS72^T and M29^T belong to distinct genomic species. The physiological, phylogenetic and genetic distance data support the description of strains JS72^T and M29^T as the type strains of novel species, for which the names *Amycolatopsis speibonae* sp. nov. (= DSM 46660^T = NRRL B-24958^T) and *Amycolatopsis roodepoortensis* sp. nov. (= DSM 46661^T = NRRL B-24959^T) are proposed, respectively.
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INTRODUCTION

The genus *Amycolatopsis*[1] belongs to the family *Pseudonocardiaceae* and contains nocardioform actinomycetes that lack mycolic acids and contain *meso*-diaminopimelic acid, arabinose and galactose in their cell wall peptidoglycan. The genus is known for its antibiotic-producing strains, producing among others the ansamycin-type antibiotic rifamycin (by *Amycolatopsis mediterranei* and *Amycolatopsis rifamycinica*)[2–4] and the glycopeptide antibiotic vancomycin (by *Amycolatopsis orientalis*).[4] Of the antibiotics produced by this genus, the ansamycin and glycopeptide classes are the most important to medicine. With the continued emergence of antibiotic resistance in bacterial pathogens, there is always a need for the discovery of new antibiotics to combat the resistance. As several members of the genus *Amycolatopsis* produce antibiotics, the genus provides an ideal source of strains to screen for such novel compounds. With the descriptions of *Amycolatopsis cihanbeyliensis*[5] and *Amycolatopsis jiangsuensis*,[6] the number of members in the genus with validly published names has risen to 62.[7] Recent advances in the taxonomy of the genus involve using phylogenetic and genetic distance analyses based on the *gyrB* and *recN* genes for distinguishing between genomic species.[8,9] Here we describe the characterization of two novel members of the genus isolated from soil samples collected from two locations in South Africa.

MATERIALS AND METHODS

Isolation

Strain M29^T was isolated from a soil sample collected from a suburban garden in Roodepoort, Gauteng Province, South Africa, and strain JS72^T from soil collected on Table Mountain above the Upper Campus of the University of Cape Town, Cape Town, South Africa. The soil sample collected from Roodepoort was subjected to microwave pretreatment. One gram of soil was vortexed in 10-ml sterile distilled water for 1 min. One milliliter of the suspension was transferred to a sterile glass Petri dish and exposed to microwave radiation (950 W output microwave oven on full power) for 20 s. After the microwave treatment the suspension was allowed to cool, serially diluted in sterile distilled water, spread-plated onto Modified Czapek Solution (MC) agar[10] and incubated at 30 °C for 21 days. The Table Mountain soil was not subjected to any pretreatment. A soil suspension was prepared as mentioned above before being serially diluted and spread-plated onto Difco Middlebrook 7H9 agar (7H9; Becton, Dickinson, Sparks, MD, USA) containing 10 mmol l^{-1} glucose (albumin-dextrose-catalase supplement omitted) and incubated at 30 °C for 14 days. After subculturing, both strains were maintained on yeast extract-malt extract agar (International *Streptomyces* Project (ISP) medium 2).[11]

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Genomic and phylogenetic analyses

Genomic DNA was extracted as previously described.[12] The rapid identification of the isolates to the genus level was achieved by 16S-rRNA gene amplification and restriction endonuclease digestion,[13] using single digestions with *Mbo*I (*Sau*3AI isoschizomer), *Vsp*I (*Asn*I isoschizomer), *Sph*I, *Kpn*I, *Hind*III, *Sal*I and *Psp*1406I. The *gyrB* gene was amplified using the 7G-gyrB-F and GgyrB-R1, and GgyrB-F1 and 7G-gyrB-R primer combinations and the standard PCR conditions described by Everest and Meyers.[8] The *recN* gene was amplified as described in Everest *et al.*[9] Strains were screened for antibiotic biosynthetic genes involved in the production of ansamycin (Type I polyketide), glycopeptide and Type II (aromatic) polyketide antibiotics as per Wood *et al.*[14] The PCR products were purified using an MSB Spin PCRapace kit (Invitek, Berlin, Germany) and sequenced. Sequence analysis, generation of concatenated gene sequences, genetic distance calculations and phylogenetic analyses were performed as detailed in Everest *et al.*[15] DNA hybridization analysis was performed as a service by the BCCM/LMG culture collection as described in Everest *et al.*,[15] with hybridizations being performed at 51 °C. Data are displayed as an average DNA–DNA hybridization value with the difference between the means of the reciprocal values given in parentheses.

Antibacterial analysis

Antimicrobial testing was performed using the standard agar overlay method as detailed in Wood *et al.*[14] Strains were stab inoculated onto agar plates of MC, 7H9, ISP 2, Hacène's medium,[16] a medium for the enhancement of antibiotic-production by *Nocardia* strains[17] and a medium for the enhancement of antibiotic production by *Streptosporangium* strains[17] and incubated at 30 °C for 7–10 days. Tests for growth inhibition were performed against *Escherichia coli* ATCC 25922, *Enterococcus faecalis* (vancomycin sensitive), *Enterococcus faecium* (clinical isolate; VanA), *Enterococcus phoeniculicola* JLB-1^T, *Mycobacterium aurum* A+ and *Staphylococcus aureus* ATCC 25923.

In addition, extractions were performed with equal volumes of organic solvents (chloroform, ethyl acetate and methanol) on the cell mass and culture filtrate fractions of 100-ml ISP 2 liquid cultures grown for 7 days at 30 °C with shaking. These extracts were concentrated 50 times and tested for activity using spot test bioautography[18] against *Bacillus subtilis* var ING, *E. faecium* (clinical isolate; VanA), *E. coli* ATCC 25922, *M. aurum* A+, *Mycobacterium bovis* strain BCG (Tokyo), *Mycobacterium smegmatis* LR222, *Mycobacterium tuberculosis* H37Rv^T (=ATCC 27294^T) and *Pseudomonas aeruginosa* ATCC 27853.

Characterization

Morphological, physiological and chemotaxonomic characterization (diagnostic diamino acid in the peptidoglycan, cell wall sugars and polar lipids) were performed as detailed in Everest *et al.*[15] The analysis of respiratory quinones was carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) on freeze-dried cells of cultures grown in ISP 2 broth at 30 °C for 3 days with moderate shaking. Fatty acid analyses were performed as a service by the BCCM/LMG Culture Collection, as per the recommendations of the commercial identification system MIDI (Microbial Identification System, Newark, DE, USA; MIDI Sherlock version 3.10; database TSBA 50 (rev 5.0)), on cells grown at 28 °C for 3 days on Tryptic Soy Agar (BBL 11768).

RESULTS AND DISCUSSION

Strains JS72^T and M29^T were determined to belong to one of the genera *Amycolatopsis*, *Pseudonocardia* or *Saccharopolyspora*, based on the analysis of the 16S-rRNA gene restriction fragment patterns.[13] BLASTN analysis,[19] based on 1410 bp of 16S-rRNA gene sequence for JS72^T and 1411 bp for M29^T, showed that both strains belong to the genus *Amycolatopsis*. The closest relatives of strain JS72^T were determined to be *Amycolatopsis thailandensis* CMU-PLA07^T (99.15% 16S-rRNA gene sequence similarity as per BLAST), *Amycolatopsis coloradensis* DSM 44225^T (99.08%) and *Amycolatopsis umgeniensis* UM16^T (99.07%). The closest relatives of strain M29^T were determined to be *Amycolatopsis decaplanina* DSM 44594^T (99.22%), *Amycolatopsis regifaucium* GY080^T (99.01%) and *Amycolatopsis keratiniphila* subsp. *nogabecina* DSM 44586^T (99.01%). Analysis of the 16S-rRNA gene sequences using EzTaxon-e[20] revealed *Amycolatopsis lurida* DSM 43134^T (99.26% 16S-rRNA gene sequence similarity) and *Amycolatopsis keratiniphila* subsp *keratiniphila* DSM 44409^T (99.12%) as additional close relatives of strains JS72^T and M29^T, with 99.48% and 99.05% similarity, respectively.

The construction of a 16S-rRNA gene maximum-likelihood phylogenetic tree[21] showed that strain M29^T grouped with *A. regifaucium* GY080^T and strain JS72^T with *A. keratiniphila* subsp *keratiniphila* DSM 44409^T (with low bootstrap support), within cluster A as defined by Everest and Meyers[8] (Figure 1). Strains JS72^T and M29^T share 99.15% 16S-rRNA gene sequence similarity.

A phylogenetic tree based on the concatenated *gyrB*-16S-rRNA gene sequences (Supplementary Figure 1) was also constructed and supported the association depicted in the 16S-rRNA gene phylogenetic tree for strain M29^T, with some minor rearrangements of the branches, but improved bootstrap support. The position of strain JS72^T, however, changed and it now formed an association with *A. umgeniensis*, but with very low bootstrap support (Supplementary Figure 1). Strain JS72^T was found to be most closely related to *Amycolatopsis alba* in the *gyrB-recN* concatenated gene phylogenetic tree (Figure 2 and Supplementary Figure 2), but the bootstrap support for this association was weak (51%). The 16S-rRNA gene sequence similarity between strain JS72^T and the type strain of *A. alba* is 98.72%. The *gyrB-recN* concatenated gene tree showed that strain M29^T clustered similarly to the other trees, but formed a close association with *A. lurida* with low (66%) bootstrap support (Figure 2).

The number of nodes with bootstrap values >70% (that is, moderate support for the depicted tree topology) in the clade containing strains JS72^T and M29^T was two in the 16S-rRNA gene tree (Figure 1), six in the *gyrB*-16S-rRNA gene tree (Supplementary Figure 1) and six in the *gyrB-recN* gene tree (Figure 2). Furthermore, the bootstrap values for the associations in Supplementary Figure 1 and Figure 2 were much higher than those in Figure 1 (the majority of which were <40%; data not shown).

Genetic distance values, based on the *gyrB* gene (1290 bp), were calculated between strains JS72^T, M29^T and all the type strains of *Amycolatopsis* species for which *gyrB* sequences are available (Supplementary Table 1), to assess whether these strains were likely to represent new species.[8] The values for strain JS72^T ranged from 0.026 to 0.213 (0.036–0.439 based on the 315 bp variable region[8]) and for strain M29^T they ranged from 0.013 to 0.210 (0.018–0.428 based on the 315-bp variable region). The *gyrB* gene-based genetic distance values between strain JS72^T and the type strains of 16S-rRNA gene cluster A (Figure 1) were all above the 0.02 threshold proposed to distinguish genomic species[8] (0.026–0.055; 0.036–0.116 based on the 315-bp variable region). The values between strain M29^T and *A. keratiniphila* subsp. *keratiniphila*, *A. keratiniphila* subsp. *nogabecina* and *A. lurida* were 0.013, 0.017 and 0.019 (0.021, 0.021 and 0.018 based on the 315-bp variable region). The values between strain M29^T and all the other members of 16S-rRNA gene cluster A were above the 0.02 threshold (0.022–0.051; 0.028–0.100 based on the 315-bp variable region). These data show that strain M29^T is likely a distinct genomic species from all *Amycolatopsis*-type strains except *A. lurida* (based on the 315-bp variable region). Thus, further evidence is needed to prove that strain M29^T is a distinct species from *A. lurida*. The *gyrB* genetic distance between JS72^T and M29^T was 0.046 (0.081 based on 315-bp variable region), above the 0.02 threshold, which indicates they are likely separate species.[8]

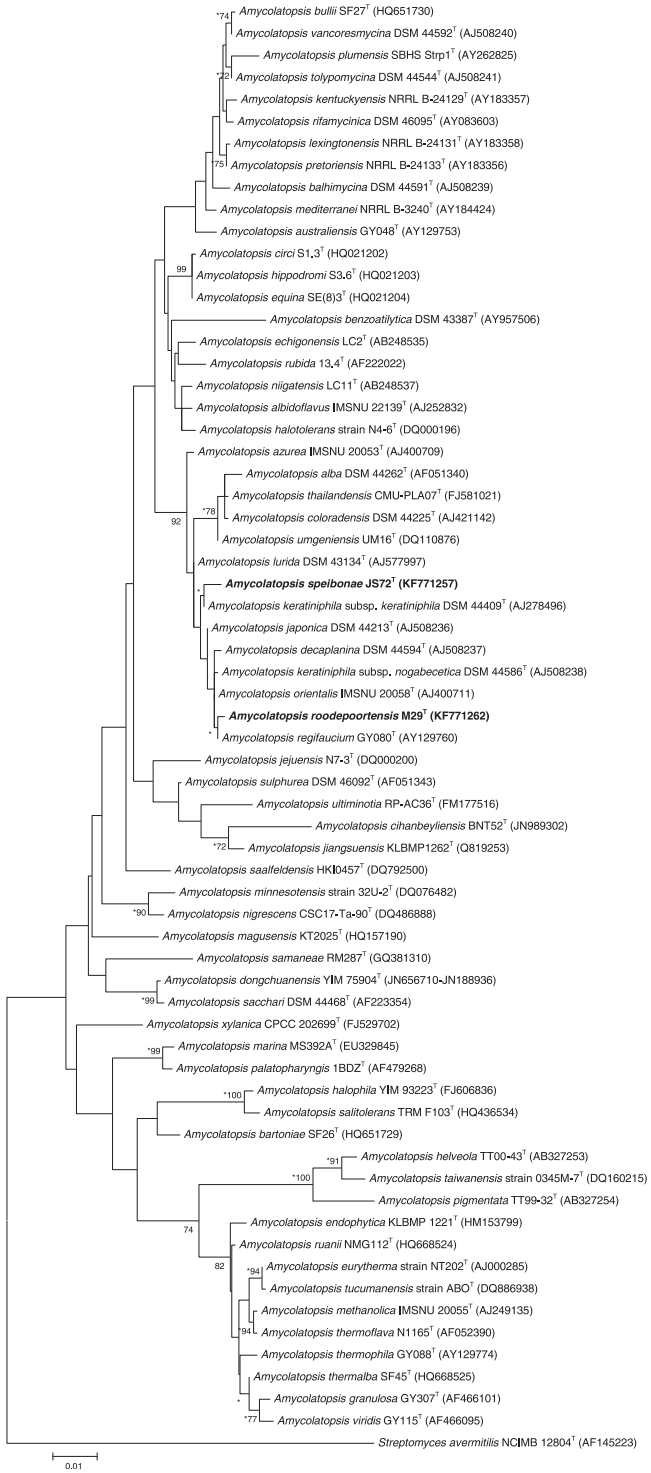

Figure 1 16S-rRNA gene phylogenetic tree showing the positions of strains JS72^T and M29^T within cluster A of the genus *Amycolatopsis*. The tree was constructed using the maximum likelihood method based on 1353 bp of common sequence. The percentage bootstrap values of 1000 replications are shown at each node (only values above 70% are shown), with asterisks (*) indicating the clades that were also formed in the trees constructed using the neighbor joining[28] and maximum parsimony[29] algorithms. Accession numbers are indicated in parenthesis after the strain numbers. The scale bar indicates 1 nucleotide substitution per 100 nucleotides.

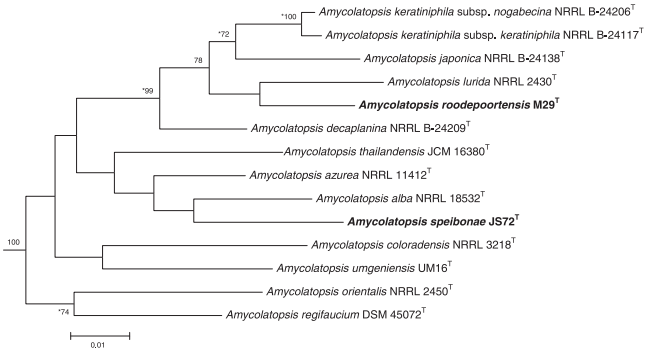

Figure 2 Subtree of the phylogenetic tree based on the concatenated *gyrB-recN* gene sequences showing the relationships of strains JS72^T and M29^T to closely related type strains. The tree forms part of a full maximum-likelihood tree, constructed based on 2520 bp of sequence for the 40 members of the genus for which *gyrB* and *recN* gene sequences are available (Supplementary Figure 2). The percentage bootstrap values of 1000 replications are shown at each node (only values above 70% are shown), with asterisks (*) indicating the clades that were also formed in the trees constructed using the neighbor joining and maximum parsimony algorithms. The strain numbers of the strains from which the *gyrB* and *recN* gene sequences were obtained are indicated. The scale bar indicates 1 nucleotide substitution per 100 nucleotides.

The *recN* gene-based genetic distance values (1231 bp) were calculated between strains JS72^T, M29^T and all type strains for which *recN* gene sequences are available (Supplementary Table 1). For strain JS72^T these genetic distances ranged from 0.056 to 0.317 (with the genetic distances against members of its 16S-rRNA gene cluster ranging from 0.056 to 0.095) and for strain M29^T the values ranged from 0.055 to 0.318 (with the genetic distances against members of 16S-rRNA gene cluster A ranging from 0.055 to 0.107). The *recN* genetic distance between strains JS72^T and M29^T was 0.095. All these values are above the proposed 0.04 threshold to distinguish novel species in the genus.[9] These data further suggest that strains JS72^T and M29^T are distinct species from all type strains of the genus *Amycolatopsis*. All *gyrB*- and *recN*-gene-based genetic distance values are presented in Supplementary Figures 3–5.

DNA–DNA hybridization experiments were performed between strains JS72^T and M29^T and their closest relatives based on 16S-rRNA gene sequence similarities. The results show that strain JS72^T shared 50(15)% DNA relatedness to *A. thailandensis* JCM 16380^T and 48(5)% to *A. lurida* NRRL 2430^T. Strain M29^T shared 29(25)% to *A. lurida* NRRL 2430^T, 26(25)% to *A. keratiniphila* subsp. *nogabecina* NRRL B-24256^T and 22(8)% to *A. decaplanina* NRRL B-24209^T. Strains JS72^T and M29^T shared 25(5)% DNA relatedness. This proves that both strains JS72^T and M29^T are distinct genomic species.[22]

Screening for antibiotic biosynthetic genes revealed the presence of the P450 monooxygenase B (*oxyB*) gene, required for the production of glycopeptide antibiotics, in both strains JS72^T (GenBank accession number: KF771261) and M29^T (KF771265). Strain JS72^T was also shown to contain the 3-amino-5-hydroxy-benzoic acid synthase gene (KF771260), required for the production of ansamycin antibiotics.

Overlay experiments showed that strain JS72^T exhibited antibacterial activity against *M. aurum* A+, *E. faecalis* (vancomycin sensitive), *E. phoeniculicola* JLB-1^T and *S. aureus* ATCC 25923, but showed no activity against *E. coli* ATCC 25922 and *E. faecium* VanA (vancomycin resistant). Strain M29^T showed activity against *M. aurum* A+, but no activity against *E. coli* ATCC 25922 and *E. faecium* VanA. Combined solvent extracts of the broth culture of

strain M29^T showed activity against *B. subtilis* var ING, *M. bovis* BCG (Tokyo), *M. aurum* A+, *M. smegmatis* LR222, *M. tuberculosis* H37Rv^T and *P. aeruginosa* ATCC 27853, but no activity against *E. coli* ATCC 25922 and *E. faecium* VanA.

Both strains JS72^T and M29^T grouped in 16S-rRNA gene cluster A of Everest and Meyers,[8] which contains most of the glycopeptide-producing *Amycolatopsis* type strains.[23] The presence of the genes for glycopeptide production in these strains and their inability to inhibit the vancomycin-resistant *E. faecium* VanA strain suggest that these strains produce a glycopeptide-type antibiotic. Further support for this is lent by the fact that strain JS72^T shows activity against a vancomycin-sensitive *E. faecalis* strain.

The results of the phenotypic characterization of strains JS72^T and M29^T are presented in Table 1 and the species descriptions. These data showed that the strains are phenotypically distinct from their closest-type strain relatives. The chemotaxonomic characteristics of the strains are presented in the species description and are consistent with membership of the genus *Amycolatopsis*.[1,24–26]

Description of *Amycolatopsis speibonae* sp. nov.

Amycolatopsis speibonae (spei.bo'nae. L. n. spes -ei hope; L. adj. bonus good; N.L. fem. adj. speibonae of good hope, to indicate Cape Town, the Cape of Good Hope, South Africa, the source of the soil from which the type strain was isolated).

Gram positive. Colonies appear convoluted with raised centers on most media. Vegetative mycelium appears cream-brown in color, fragmenting into short rod-shaped elements in both liquid and agar cultures. Aerial mycelium appears white on ISP 4. No diffusible

Table 1 Phenotypic differences between strains M29^T, JS72^T and closely related *Amycolatopsis*-type strains

Test	*JS72^T*	*M29^T*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Color of														
Spore mass (on ISP 4)	Wh	Cr	Wh	Wh	Wh	Wh	Wh	Wh	Cr	Cr-Wh	Cr-Wh	Cr	Wh	Wh
Diffusible pigments	−	−	−	−	Yel	−	−	−	−	−	−	−	−	−
H_2S production	−	+	+	−	+	+	+	+	+	+	+	+	+	+w
Nitrate reduction	+	−	−	+	−	+	−	+	+	−	+	−	−	+
Degradation of														
Allantoin	+w	−	+w	−	−	−	−	−	−	−	−	−	−	+
Guanine	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Urea	+	+	−	−	+	+	+	+	+	+	−	−	+	+
Xanthine	−	+	+w	+	+w	+	+	+w	−	+	+w	+	+	+
Xylan	−	+w	−	−	−	−	−	−	−	−	+w	+w	−	−
Growth at														
pH 4.3	+	+	−	−	+w	++	−	+	+	+w	−	−	++	+
pH 10	++	++	−	−	+	++	+w	++	++	+	+w	−	++	+
37 °C	−	+	−	+w	+	++	+	+	++	+	++	++	+	−
Growth in the presence of														
2% (w/v) NaCl	++	++	−	+	+	++	+w	++	++	+	−	+	+	+
4% (w/v) NaCl	+	+	−	+	+	++	+w	+	+	+w	−	−	+	+
7% (w/v) NaCl	−	+w	−	+	+w	+w	−	+w	−	−	−	−	+w	+
Utilization as sole carbon source														
Adonitol	+	++	++	+	−	−	++	+w	+	+	++	−	−	++
L(+)-Arabinose	+	++	−	++	−	++	+	+	++	+	+	+	−	+
Lactose	+w	+	+	+	−	++	+	+	++	+w	+	+w	++	+
D(+)-Melibiose	+w	−	+	+w	−	++	++	+	−	−	+w	−	+w	++
Raffinose	+w	−	+w	+w	−	+w	+	+w	−	−	+w	−	+w	+
L(+)-Rhamnose	−	−	−	−	−	−	−	−	−	−	++	−	−	−
Sodium citrate	+w	+w	+w	+w	+w	+w	+w	+w	+w	+w	+w	+w	−	+
Sucrose	−	+w	−	+w	−	+w	−	+w	+w	−	−	+w	+w	−
Utilization of sole nitrogen source														
DL-α-amino-n-butyric acid	+	+	+	+w	−	+w	+	+w	+w	+w	+w	+w	+	+
L-Cysteine	+w	+w	+w	+w	−	+w	+w	+w	+w	+w	+w	+w	+w	+
L-4-Hydroxyproline	+w	+w	+w	+w	−	+w	++	+w	+	−	+w	+w	+	+
L-Methionine	−	+w	+w	−	−	+w	+w	+w	+w	−	+w	−	+w	+w
L-Phenylalanine	+w	+	+	+w	−	+w	+	++	+	+w	+	w	+	+
L-Threonine	+	+w	+w	+	−	+w	+w	+	++	+w	+	+w	+w	++
L-Valine	+w	+	+	+	−	+w	+	+	+	+w	+	+w	+w	++

Reference strains: 1, *A. alba* NRRL 18532^T; 2, *A. azurea* NRRL 11412^T; 3, *A. coloradensis* NRRL 3218^T; 4, *A. decaplanina* NRRL B-24209^T; 5, *A. japonica* NRRL B-24138^T; 6, *A. keratiniphila* subsp. *keratiniphila* NRRL B-24117^T; 7, *A. keratiniphila* subsp. *nogabecina* NRRL B-24206^T; 8, *A. lurida* NRRL 2430^T; 9, *A. orientalis* NRRL 2450^T; 10, *A. regifaucium* DSM 45072^T; 11, *A. thailandensis* JCM 16380^T; 12, *A. umgeniensis* UM16^T. All data were determined in this study. Symbols: ++, strong positive; +, positive; +w, weakly positive; −, negative; Cr, cream; Wh, white; Yel, yellow.

pigments are produced. Melanin is not produced on peptone-yeast extract-iron agar (ISP 6) or tyrosine agar (ISP 7). Grows at 30 °C, but not at 37 °C. Grows at pH 4.3, 7 and 10 as well as in the presence of up to 4% (w/v) NaCl, but not in the presence of 7% (w/v) NaCl. Catalase positive. Oxidase negative. Nitrate is reduced to nitrite. Does not produce H_2S. Starch is not hydrolyzed. Casein, gelatin, hypoxanthine, Tween 80, L-tyrosine and urea are degraded. Allantoin is weakly degraded. Adenine, guanine, xanthine and xylan are not degraded. Utilizes adonitol, L(+)-arabinose, D(+)-cellobiose, D(–)-fructose, D(+)-glucose, *meso*-inositol, D(–)-mannitol, D(+)-mannose and D(+)-xylose as sole carbon sources. Weakly utilizes raffinose, D(+)-melibiose, α-lactose, salicin, sodium acetate and sodium citrate. Unable to utilize inulin, L(+)-rhamnose and sucrose as sole carbon sources. Utilizes DL-α-amino-n-butyric acid, L-asparagine, L-histidine, potassium nitrate and L-threonine as sole nitrogen sources, with weak growth on L-arginine, L-cysteine, L-4-hydroxyproline, L-phenylalanine, L-serine and L-valine. Unable to utilize L-methionine as a sole nitrogen source. Contains a type IV cell wall (*meso*-diaminopimelic acid, arabinose and galactose in the cell wall).[27] Mycolic acids are absent. The polar lipid profile includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylinositol-mannoside, two unidentified phospholipids, two unidentified aminolipids, two unidentified glycolipids, an unidentified phosphoglycolipid and an unidentified aminophospholipid (Supplementary Figure 6). The predominant menaquinone is MK-9(H_4) (85%), with minor amounts of MK-8(H_4) (11%). The major fatty acids of the type strain are i-$C_{15:0}$ (17.36%), $C_{17:1}\omega8c$ (16.93%), $C_{17:0}$ (13.45%), ai-$C_{15:0}$ (11.14%), $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (9.44%) and i-$C_{16:0}$ (9.7%), with minor amounts of $C_{15:1}\omega6c$ (7.42%), i-$C_{14:0}$ (6.5%), ai-$C_{17:0}$ (3.28%), $C_{17:1}\omega6c$ (1.7%), $C_{13:0}$ (1.54%) and $C_{16:0}$ (1.53%).

Antibacterial activity is exhibited against *E. phoeniculicola* JLB-1^T, a vancomycin-sensitive *Enterococcus* sp., *M. aurum* A+ and *S. aureus* ATCC 25923.

The type strain, JS72^T (= DSM 46660^T = NRRL B-24958^T), was isolated from soil from Table Mountain above the Upper Campus of the University of Cape Town, Cape Town, South Africa.

Description of *Amycolatopsis roodepoortensis* sp. nov.

Amycolatopsis roodepoortensis (roo.de.poort.en'sis. N.L. fem. adj. *roodepoortensis*, pertaining to Roodepoort, a suburb in Gauteng province of South Africa, the source of the soil from which the type strain was isolated).

Gram positive. Colonies appear convoluted with raised centers on most media. Vegetative mycelium appears cream-brown in color, fragmenting into short rod-shaped elements in both liquid and agar cultures. Aerial mycelium appears cream on ISP 4. No diffusible pigments are produced. Melanin is not produced on peptone-yeast extract-iron agar (ISP 6) or tyrosine agar (ISP 7). Grows at 30 °C and at 37 °C. Grows at pH 4.3, 7 and 10 as well as in the presence of up to 7% (w/v) NaCl. Catalase positive. Oxidase negative. Nitrate is not reduced to nitrite. Produces H_2S. Starch is not hydrolyzed. Casein, gelatin, hypoxanthine, Tween 80, L-tyrosine, urea and xanthine are degraded. Xylan is weakly degraded. Adenine, allantoin and guanine are not degraded. Utilizes adonitol, L(+)-arabinose, D(+)-cellobiose, D(–)-fructose, D(+)-glucose, *meso*-inositol, lactose, D(–)-mannitol, D(+)-mannose, salicin and D(+)-xylose as sole carbon sources. Weakly utilizes sodium acetate, sodium citrate and sucrose. Unable to utilize inulin, D(+)-melibiose, raffinose and L(+)-rhamnose as a sole carbon source. Utilizes DL-α-amino-n-butyric acid, L-arginine, L-asparagine, L-histidine, L-serine, L-valine and L-phenylalanine as sole nitrogen sources, with weak growth on L-cysteine, L-4-hydroxyproline, L-methionine, potassium nitrate and L-threonine. Contains a type IV cell wall (*meso*-diaminopimelic acid, arabinose and galactose in the cell wall).[27] Mycolic acids are absent. The polar lipid profile includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, two unidentified phospholipids, an unidentified aminolipid, six unidentified glycolipids, an unidentified aminophospholipid, three unidentified aminoglycolipids and an unidentified phosphoglycolipid (Supplementary Figure 7). The predominant menaquinone is MK-9(H_4) (84%), with minor amounts of MK-8(H_4) (12%) and MK-9(H_2) (3%). The major fatty acids of the type strain are $C_{17:1}\omega8c$ (22.99%), i-$C_{16:0}$ (22.55%), $C_{17:0}$ (9.5%) and $C_{17:1}\omega6c$ (9%), with minor amounts of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (7%), i-$C_{15:0}$ (6.75%), $C_{15:1}\omega6c$ (6.72%), i-$C_{14:0}$ (6.44%), ai-$C_{15:0}$ (1.99%), $C_{16:0}$ (1.85%), ai-$C_{17:0}$ (1.81%), $C_{15:0}$ 2-OH (1.2%), i-$C_{17:0}$ (0.76%), $C_{18:1}\omega9c$ (0.53%), $C_{13:0}$ (0.44%) and $C_{14:0}$ (0.38%). Antibacterial activity is exhibited against *B. subtilis* var ING, *M. bovis* BCG (Tokyo), *M. aurum* A+, *M. smegmatis* LR222, *M. tuberculosis* H37Rv^T and *P. aeruginosa* ATCC 27853.

The type strain, M29^T (= DSM 46661^T = NRRL B-24959^T), was isolated from soil from a suburban garden in Roodepoort, Gauteng Province, South Africa.

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ACCESSION NUMBERS

The GenBank accession numbers for the 16S-rRNA, *gyrB* and *recN* gene sequences of strain JS72^T are KF771257, KF771258 and KF771259, respectively, and those for strain M29^T are KF771262, KF771263 and KF771264, respectively.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)