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TAXONOMIC DESCRIPTION

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Classification of *Actinoplanes* sp. ATCC 33076, an actinomycete that produces the glycolipodepsipeptide antibiotic ramoplanin, as *Actinoplanes ramoplaninifer* sp. nov.

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Abstract

Strain ATCC 33076, which produces the antibiotic ramoplanin, was isolated from a soil sample collected in India, and it was classified as a member of the genus *Actinoplanes* on the basis of morphology and cell-wall composition. A phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain forms a distinct clade within the genus *Actinoplanes*, and it is most closely related to *Actinoplanes deccanensis* IFO 13994^T (98.71 % similarity) and *Actinoplanes atraurantiacus* Y16^T (98.33 %). The strain forms an extensively branched substrate mycelium; the sporangia are formed very scantily and are globose with irregular surface. Spores are oval and motile. The cell wall contains *meso*-diaminopimelic acid and the diagnostic sugars are xylose and arabinose. The predominant menaquinone is MK-9(H_6), with minor amounts of MK-9(H_4) and MK-9(H_2). Mycolic acids are absent. The diagnostic phospholipids are phosphatidylethanolamine, hydroxyphosphatidylethanolamine and phosphatidylglycerol. The major cellular fatty acids are anteiso-$C_{17:0}$ and iso-$C_{16:0}$, followed by iso-$C_{15:0}$ and moderate amounts of anteiso-$C_{15:0}$, iso-$C_{17:0}$ and $C_{18:1}\omega 9c$. The genomic DNA G+C content is 71.4 mol%. Significant differences in the morphological, chemotaxonomic and biochemical data, together with DNA–DNA relatedness between strain ATCC 33076 and closely related type strains, clearly demonstrated that strain ATCC 33076 represents a novel species of the genus *Actinoplanes*, for which the name *Actinoplanes ramoplaninifer* sp. nov. is proposed. The type strain is ATCC 33076^T (=DSM 105064^T=NRRL B-65484^T).

The genus *Actinoplanes* was first described by Couch [1] as a member of the family *Micromonosporaceae* and with *Actinoplanes philippinensis* as the type species. A comprehensive description of the genus has been recently given by Vobis *et al.* [2]. Members of the genus *Actinoplanes* are aerobic, Gram-stain-positive, non-acid-fast actinomycetes that can form extensively branched substrate mycelium on various agar media, but aerial mycelia are usually absent or rudimentarily developed. These organisms develop differently shaped or irregular sporangia that contain motile sporangiospores. The peptidoglycan of the cell wall contains *meso*- and/or 3-hydroxy-diaminopimelic acid, and the diagnostic sugar of whole-cell hydrolysates is xylose; galactose and/or arabinose are also present [3, 4]. The polar lipid profile is characterized by the presence of phosphatidylethanolamine as diagnostic phospholipid and the absence of phosphatidylcholine and amino-containing phosphoglycolipids, corresponding to the phospholipid type PII of Lechevalier *et al.* [5]. The whole polar lipid composition may also contain a highly hydrophilic glycolipid and further unknown or uncharacterized lipids and glycolipids [2, 6]. Di-, tetra- and hexa-hydrogenated menaquinones with nine isoprene units are predominant isoprenologues [6, 7]. Iso/anteiso-branched and monounsaturated fatty acids and/or *cis*-9, 10-octadecanoic acid are often present, though their proportions may vary considerably between the species [2, 6].

At the time of writing, the genus comprises 45 species with validly published names, including the recently described *Actinoplanes lichenis* [8] and *Actinoplanes subglobosus* [9]. Members of the genus *Actinoplanes* are widespread in nature, and have been isolated from a wide range of natural habitats such as leaf litter, soil, including terrestrial and rhizospheric soil, as well as lake and river water, and animal and vegetable debris [10–13]. They were also isolated from a lichen sample collected from tree bark in Thailand [8] and from paper wasp nests [14]. Additionally, they were found as endophytes in the stems and roots of rice [15]. *Actinoplanes* strains have shown a high capacity to produce

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One supplementary table and three supplementary figures are available with the online Supplementary Material.

antibiotics [2, 16]. The amino acid derivatives are predominant and some of them are of clinical relevance [17, 18].

In the 1970s, Lepetit Research Laboratories screened for antibiotic production several thousands of *Actinoplanes* spp. – mainly isolated from Italian and Indian soil samples – and discovered actagardine, teicoplanin, lipiarmycin (fidaxomicin) and ramoplanin from *Actinoplanes liguriensis*, *A. teichomyceticus*, *A. deccanensis* and *Actinoplanes* sp. ATCC 33076, respectively [16, 19]. Strain ATCC 33076 was isolated from a soil sample collected at Vaghalbod village, Indore, India. It produces the glycolipodepsipeptide antibiotic ramoplanin (initially named A/16686) [20]. Ramoplanin is an inhibitor of peptidoglycan synthesis, which acts by sequestering lipid II. It attracted the attention of medical bacteriologists because of its excellent bactericidal activity against important pathogens, including Gram-positive bacteria such as *Enterococcus faecium*, *Clostridum difficile* and meticillin-resistant *Staphylococcus aureus* strains [21]. The systemic use of ramoplanin has been so far prevented by its low local tolerability when injected intravenously. Recently, it was orally tested in clinical trials for the prophylaxis and cure of *C. difficile* infections [22]. The ramoplanin-producing strain was originally classified as a member of the genus *Actinoplanes* [20] on the basis of morphology and cell-wall composition. Notwithstanding its pharmaceutical relevance as the producer of ramoplanin, strain ATCC 33076 is still poorly taxonomically characterized. In this study, we performed a polyphasic taxonomic analysis of strain ATCC 33076, and propose that it represents a novel species of the genus *Actinoplanes*.

Strain ATCC 33076 was isolated by employing a method based on the fact that, unlike streptomycetes, *Actinoplanes* species produce motile spores. A soil sample was dispersed in water containing 10 µg novobiocin ml^{-1} and settled by centrifugation. Motile spores migrated into the supernatant. The supernatant was collected after 4 h at room temperature, and then plated on Czapek glucose agar containing 10 µg novobiocin ml^{-1}. Colonies of actinomycetes appeared on the agar after incubation at 28 °C for 2–3 weeks and were transferred, using sterile toothpicks, to plates of ISP 3 medium [23]. The pure culture was stored as 20 % (v/v) glycerol suspensions at –80 °C and as lyophilized cells for long-term preservation.

Genomic DNA of strain ATCC 33076 was extracted as described in Kieser *et al.* [24] with some modifications. 16S rDNA was amplified by PCR according to the protocol of the Bact 16S Service of BMR Genomics (Padova, Italy) (http://www.bmr-genomics.it/ngs2015/?s=Bact+16S) using universal primers and Platinum *Taq* High Fidelity (Fisher Scientific). The PCR product was sequenced by BMR Genomics using universal primers. BLAST analysis was used to compare the nearly complete 16S rRNA gene sequence of strain ATCC 33076 with those of representatives of the genus *Actinoplanes* retrieved from public nucleotide databases. The 16S rDNA sequence similarity between strain ATCC 33076 and strains of related species was calculated using the EzTaxon-e server (http://www.ezbiocloud.net/taxonomy; [25]). A multiple sequence alignment of strain ATCC 33076 and other related taxa was performed by using CLUSTAL W version 2.0 [26]. Phylogenetic trees were reconstructed using the neighbour-joining [27] and maximum-likelihood [28] algorithms by MEGA software version 6.0 [29]. Topology of phylogenetic trees was evaluated with bootstrap analysis based on 1000 resamplings [30] to determine the confidence values of branches of the phylogenetic tree. A distance matrix was generated using Kimura's two-parameter model [31]. All positions containing gaps and missing data were eliminated from the dataset.

Comparison of the almost-complete 16S rRNA gene sequence (1409 nt) of strain ATCC 33076 with those of type strains of related species with validly published names by using the EzTaxon-e server indicated that strain ATCC 33076 is a member of the genus *Actinoplanes*. The most closely related type strains were *A. deccanensis* IFO 13994^T (98.71 % similarity; 18 nucleotide differences in 1400 positions) and *A. atraurantiacus* Y16^T (98.33 %; 23 nucleotide differences in 1380 positions). Levels of 16S rRNA gene sequence similarity to the type strains of other species of the genus *Actinoplanes* were lower than 98.0 %. The neighbour-joining phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences shows that strain ATCC 33076 forms a distinct cluster with *A. deccanensis* IFO 13994^T and *A. atraurantiacus* Y16^T, these last two forming a phyletic line. The neighbour-joining tree is in agreement with the maximum-likelihood tree, where strain ATCC 33076 belongs to the cluster including *A. deccanensis* IFO 13994^T and *A. atraurantiacus* Y16^T (Fig. S1, available in the online Supplementary Material).

The morphological characteristics of strain ATCC 33076 were observed by light (Zeiss Primo Star) and scanning electron (SEM-FEG XL-30; Philips) microscopy using cultures grown on potato agar medium in black-tea extract [16] at 28 °C for 2 months. For observation of spore motility, mature sporangia formed on agar plates were washed with pre-warmed 10 mM potassium phosphate buffer (pH 7.0) at 30 °C for 30 min and spores were collected and analysed with a phase contrast microscope following the protocol previously described by Uchida *et al.* [32]. The morphology and dehiscence of sporangia were observed by examining gold-coated dehydrated specimens with a scanning electron microscope [33]. Culture characteristics of strain ATCC 33076 in parallel with those of *A. deccanensis* DSM 43806^T (=IFO 13994^T) and *A. atraurantiacus* DSM 45850^T (=Y16^T) were determined after incubation at 28 °C for 14–21 days on various ISP media [23] in addition to several media recommended by Waksman [34]. The agar media used for morphological and culture studies were: yeast extract–malt agar (ISP 2), oatmeal agar (ISP 3), inorganic salts–starch agar (ISP 4), glycerol–asparagine agar (ISP 5), peptone–yeast extract–iron agar (ISP 6), tyrosine agar (ISP 7), oatmeal agar (according to Waksman), nutrient agar, potato agar, Bennett's agar, Hickey–Tresner agar, glucose–asparagine agar, Czapek glucose agar, skimmed milk agar, peptone glucose

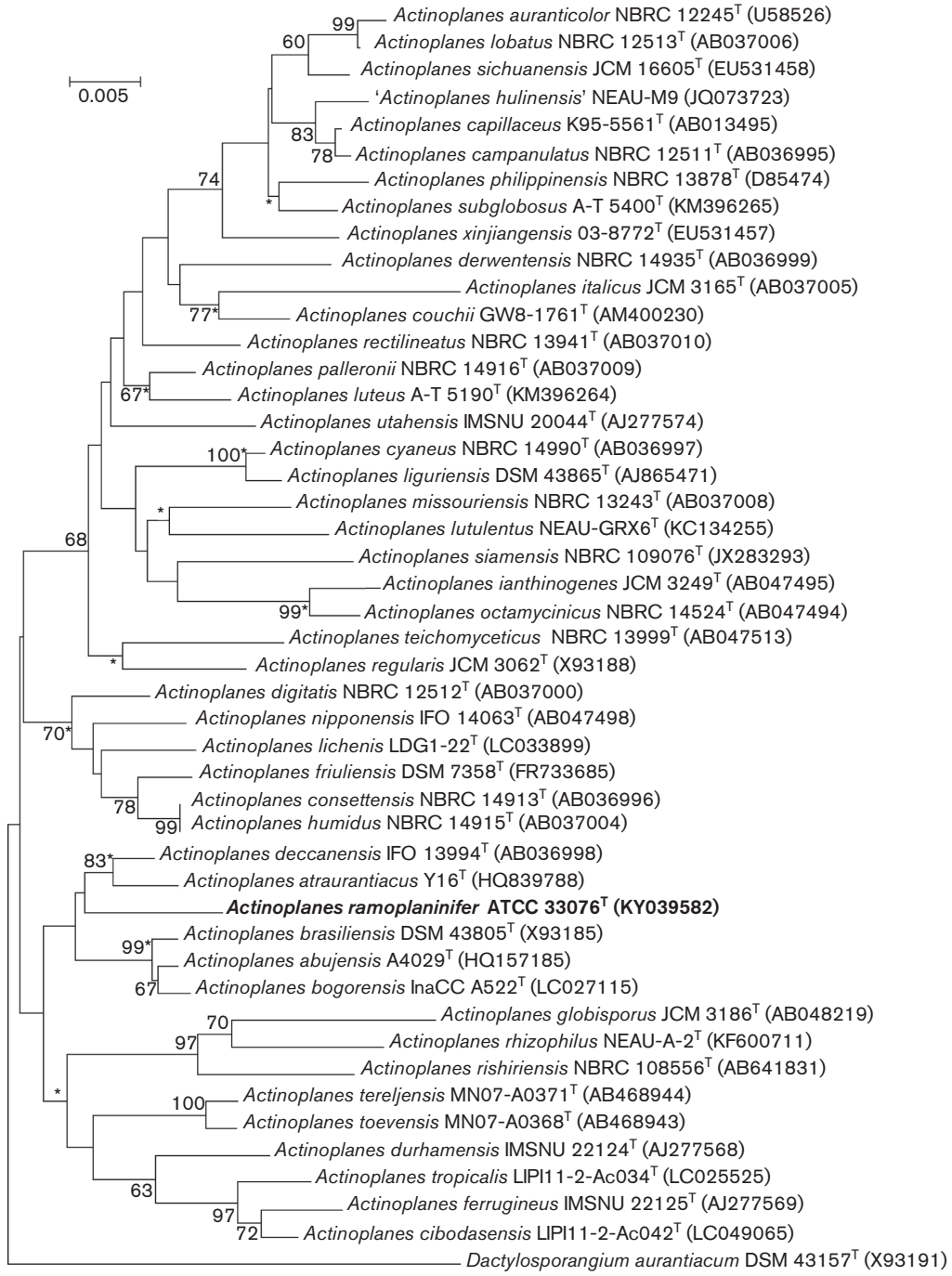

Fig. 1. Neighbour-joining tree based on almost complete 16S rRNA gene sequences showing the relationship between strain ATCC 33076 and other species of the genus *Actinoplanes*. *Dactylosporangium aurantiacum* DSM 43157^T was used as an outgroup. The phylogenetic analysis compared 1344 sites, following the complete deletion of gaps and missing data. Asterisks denote branches that were also recovered from the maximum-likelihood tree. Numbers at branch points indicate bootstrap percentages (based on 1000 replications); only values above 50.0 % are shown. Bar, 0.005 substitutions per nucleotide position.

agar. Colours were determined according to Maerz and Paul [35]. The ranges of temperature (22–42 °C), pH (3.0–12.0) and NaCl concentration (0–5.0 %, w/v) for growth of strain ATCC 33076 were examined on Hickey–Tresner agar plates for 14–21 days. Starch hydrolysis was checked on ISP 4 medium. Gelatin liquefaction was evaluated on glucose–peptone–gelatin medium (2.0 % glucose, 0.5 % peptone, 20.0 % gelatin, pH 7.0). Peptonization and coagulation of

Table 1. Comparison of culture characteristics of strain ATCC 33076 and type strains of the most closely related species of the genus *Actinoplanes* with validly published names

Strains: 1, ATCC 33076; 2, *A. deccanensis* DSM 43806^T; 3, *A. atraurantiacus* DSM 45850^T. G, Growth (+++, good; ++, moderate; +, poor; –, no growth); AM, aerial mycelium; SM, substrate mycelium; SP, soluble pigment.

Medium		1	2	3
ISP 2	G	+++	+++	+++
	AM	None	None	None
	SM	Light brown	Light orange	Light yellow to dark orange
	SP	None	None	None
ISP 3	G	+	+	+++
	AM	None	None	None
	SM	Light orange	Light amber	Light yellow to dark orange
	SP	None	None	None
ISP 4	G	++	+++	+++
	AM	None	None	None
	SM	Orange	Orange	Light yellow to dark orange
	SP	None	None	None
ISP 5	G	+	++	++
	AM	None	None	None
	SM	Whit ish	Orange	Light yellow to dark orange
	SP	None	None	None
ISP 6	G	+	++	++
	AM	None	None	None
	SM	Whitish to light brown	Light orange	Light yellow to dark orange
	SP	None	None	None
ISP 7	G	+	+++	+++
	AM	None	None	None
	SM	Brown	Amber to light brown	Light yellow to dark orange
	SP	None	Yes (brown)	Yes (Pink)
Nutrient agar	G	++	++	+
	AM	None	None	None
	SM	Orange	Light orange	Light orange to pinkish
	SP	None	None	None
Hickey–Tresner agar	G	+++	+++	++
	AM	None	None	None
	SM	Orange	Light orange to pinkish	Cream to light orange
	SP	None	None	None
Glucose–asparagine agar	G	+	++	++
	AM	None	None	None
	SM	Light orange	Cream to light orange	White waxy
	SP	None	None	None
Potato agar	G	+++	+++	++
	AM	None	None	None
	SM	Amber to brown	Pale orange	Light orange to pinkish
	SP	None	None	None
Peptone glucose agar	G	+++	++	++
	AM	None	None	None
	SM	Orange	Orange	Orange
	SP	None	None	None

milk were assayed in 10.0 % (w/v) skimmed milk broth (Difco). Degradation of other compounds was examined using Bennett's agar according to Goodfellow *et al.* [36]. Production of H_2S and melanin pigment was determined on ISP 6 and ISP 7 media, respectively. Nitrate reduction was observed using nitrate broth (Difco). Carbon source

utilization was tested using carbon source utilization medium (ISP 9) [23] supplemented with a final concentration of 1 % of the tested carbon source.

The morphological and culture characteristics of strain ATCC 33076 are consistent with those of members of the genus *Actinoplanes*. Strain ATCC 33076 showed good growth on ISP 2, oatmeal agar (according to Waksman), Hickey–Tresner agar, Bennett's agar, potato agar, skimmed milk agar and peptone glucose agar, and moderate growth on ISP 4, Czapeck glucose agar, and nutrient agar. Strain ATCC 33076 formed a flexuous and branched substrate mycelium. Fragmentation of substrate mycelium was never observed. As reported in Table 1, the colour of the substrate mycelium varied from brown to amber and orange according to the medium used. The strain did not produce soluble pigment in any of the media used. Aerial mycelium was always absent. The sporangia formed very scantly and irregularly on potato agar in black-tea extract. They were globose, directly formed on substrate mycelium (Fig. 2a), then emerging with an irregular surface and a diameter ranging from 5.0 to 10.0 µm when observed by scanning electron microscopy (Fig. 2b). Spore release was observed after rupture of the wall of the sporangium envelope (Fig. 2c, d). The oval spores were motile, as observed by phase-contrast optical microscopy.

Strain ATCC 33076 tolerated temperatures ranging from about 22 to 42 °C with an optimum between 28 and 37 °C. The strain grew at pH 6.0–11.0. Strain ATCC 33076 tolerated up to 1.0 % (w/v) NaCl (Table 2). Starch hydrolysis, gelatin liquefaction, milk coagulation, nitrate reduction, casein degradation and hydrogen sulfide production were positive. Milk peptonization, cellulose decomposition and melanin production were negative. Culture and physiological characteristics of strain ATCC 33076 that differentiate it from closely related species are reported in Tables 1 and 2, and indicated in detail in the species description.

For a comparative analysis of antibiotic production, strain ATCC 33076 and the closest strains *A. deccanensis* DSM 43806^T and *A. atraurantiacus* DSM 45850^T were cultivated in VB (vegetative) and PB (production) media, which were previously optimized for ramoplanin production [37]. The strains grew for 192 h on a rotary shaker at 200 r.p.m. and 28 °C. Cultures were sampled every 24 h. Biomass was measured as dry weight after harvesting the mycelium by centrifugation for 10 min at 4000 ***g*** and then dehydrating the pellet for 24 h in a 50 °C oven. The antibiotic ramoplanin was extracted by mixing 1 vol. culture with 3 vols acetone and 2 vols water and brought to pH 2.2 with 1 N HCl following method reported in Brunati *et al.* [37].

As shown in Fig. S2(a), in the production medium PB – previously optimized for ramoplanin production by strain ATCC 33076 – *A. atraurantiacus* DSM 45850^T and *A. deccanensis* DSM 43806^T grew to a lesser extent and better than strain ATCC 33076, respectively, but neither of them produced any trace of the antibiotic ramoplanin. In contrast, a significant amount of this glycolipodepsipeptide antibiotic was detectable from cultures of strain ATCC 33076 (Fig. S2b).

Biomass for chemotaxonomic study was prepared by growing strain ATCC 33076 in ISP 2 broth [23] at 200 r.p.m. for 5 days at 30 °C; cells were harvested by centrifugation, washed twice in distilled water, recentrifuged and freeze-dried. Whole-cell amino acids and sugars were prepared according to Lechevalier and Lechavalier [4] and analysed by TLC [38]. The presence of mycolic acids was checked by the method of Minnikin *et al.* [39]. Isoprenoid quinones were extracted and purified using the methods of Collins *et al.* [40] and analysed by HPLC [41]. Polar lipids and fatty acids were extracted and analysed by the Identification Service of the Leibniz-Institut DSMZ (Deutsche Sammlungvon Mikroorganismen und Zellkulturen, Braunschweig, Germany). Briefly, polar lipids were separated by two-dimensional silica gel TLC (Macherey-Nagel). The first direction was developed in chloroform:methanol:water (65 : 25 : 4, v/v/v), and the second in chloroform:methanol:acetic acid: water (80 : 12 : 15 : 4, v/v/v/v). Total lipid material was detected using molybdatophosphoric acid, and specific functional groups using spray reagents specific for defined

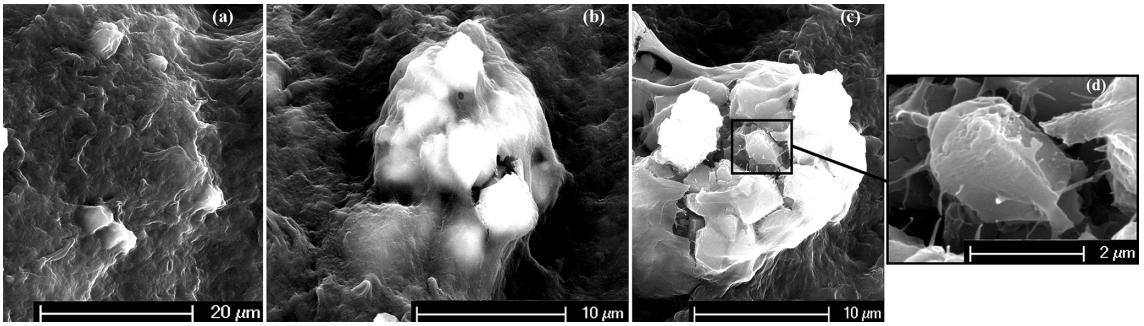

Fig. 2. Scanning electron micrographs showing early formation of sporangia (a), mature sporangium (b), sporangium dehiscence (c) and spore morphology (d). The strain was grown on potato agar in black-tea extract at 30 °C for 60 days. Bars: 20 µm (a), 10 µm (b, c) and 2 µm (d).

Table 2. Physiological properties that differentiate strain ATCC 33076 from the type strains of the most closely related species of the genus *Actinoplanes*

Strains: 1, ATCC 33076; 2, *A. deccanensis* DSM 43806^T; 3, *A. atraurantiacus* DSM 45850^T. +, Growth/degradation; −, absence of growth/degradation; w, weakly positive.

Characteristics	1	2	3
Temperature growth (°C)	22–42	26–42	25–37
pH range	6–11	5–10	6–9
NaCl tolerance (% w/v)	1	3	3
Milk coagulation	+	−	−
H_2S production	+	−	−
Casein hydrolysis	+	−	+
Tyrosinase reaction	−	+	−
Growth on sole carbon source			
L-Arabinose	+	+	−
Cellobiose	+	−	+
D-Fructose	+	−	+
Lactose	−	+	+
D-Mannitol	−	−	w
Salicin	+	−	+

functional groups according to Tindall *et al.* [42]. For the extraction of whole-cell fatty acids, cells were grown in trypticase soy broth at 200 r.p.m. for 5 days at 28 °C. After harvesting by cellulose membrane filtration (0.45 mm), the wet biomass was extracted using minor modifications [43] of the method of Miller [44]. Analyses were carried out as described by Kroppenstedt *et al.* [45] and fatty acid methyl ester peaks were separated by the Microbial Identification System (MIDI; Microbial ID) and analysed using the TSBA database, version 40.

Strain ATCC 33076 contained *meso*-diaminopimelic acid as the cell-wall diamino acid and the whole-cell sugars were xylose and arabinose (type D). Mycolic acids were not detectable. As shown in Fig. S3, the diagnostic phospholipids were phosphatidylethanolamine, hydroxyphosphatidylethanolamine and phosphatidylglycerol. The predominant menaquinone of the strain was MK-9(H_6) (74.6 %), while minor amounts of MK-9(H_2) (7.5 %) and MK-9(H_4) (13.4 %) were also detectable. The major cellular fatty acids were anteiso-$C_{17:0}$ (22.6 %) and iso-$C_{16:0}$ (22.2 %), followed by iso-$C_{15:0}$ (14.2 %); moderate amounts of anteiso-$C_{15:0}$ (6.0 %), iso-$C_{17:0}$ (5.9 %) and $C_{18:1}\omega9c$ (5.3 %) were also present. Table S1 reports the difference in fatty acid composition between strain ATCC 33076 and closely related species.

The DNA G+C content of strain ATCC 33076 was determined by the Identification Service of the Leibniz-Institut DSMZ using the following procedure. Cells were disrupted with a Constant Systems TS 0.75 kW (IUL Instruments) and DNA was purified on hydroxyapatite according to Cashion *et al.* [46]. The DNA was hydrolysed with P1 nuclease and the nucleotides were dephosphorylated with bovine alkaline phosphatase [47]. The resulting deoxyribonucleosides were analysed by HPLC [48]. Lambda DNA and three DNAs with published genome sequences representing a G+C range of 43.0–72.0 mol% were used as standards. DNA G+C contents were calculated from the ratio of deoxyguanosine and thymidine according to the method of Mesbah and Whitman [47].

The G+C content of the DNA of strain ATCC 33076 was 71.4±0.3 mol%.

According to phenotypic and genotypic results, strain ATCC 33076 is different from the type strains of closely related species. To confirm this, DNA–DNA hybridization between strain ATCC 33076 and the closest strains *A. deccanensis* DSM 43806^T and *A. atraurantiacus* DSM 45850^T was determined by the Identification Service of the Leibniz-Institut DSMZ. Cells were disrupted by using a Constant Systems TS 0.75 kW (IUL Instruments) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* [46]. DNA–DNA hybridization was carried out as described by De Ley and Tijtgat [49] with the modifications reported by Huss *et al.* [50] using a model Cary 100 Bio UV/Vis-spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). DNA–DNA relatedness values were examined from two independent determinations. Strain ATCC 33076 showed DNA–DNA relatedness of 47.4±5.6 % to *A. deccanensis* DSM 43806^T and 25.5±0.5 % to *A. atraurantiacus* DSM 45850^T: both values are clearly below the 70 % threshold value for the definition of bacterial species [51].

On the basis of the data from our polyphasic study, strain ATCC 33076 differs from closely related species of the genus *Actinoplanes*; therefore, strain ATCC 33076 represents a novel species, for which the name *Actinoplanes ramoplaninifer* sp. nov. is proposed.

DESCRIPTION OF *ACTINOPLANES RAMOPLANINIFER* SP. NOV.

Actinoplanes ramoplaninifer (ra.mo.pla.ni′ni.fer. N.L. neut. n. *ramoplaninum* ramoplanin; suff. *–fer* (from L. v. *fero*) one that carries; N.L. masc n. *ramoplaninifer* ramoplanin carrier).

Aerobic, Gram-stain-positive actinomycete that forms extensively branched, brown, amber to orange substrate mycelium on ISP 2, oatmeal agar (according to Waksman), Hickey–Tresner agar, Bennett's agar, potato agar, skimmed milk agar and peptone glucose agar. Aerial mycelium is absent. Globose sporangia form very scantly and irregularly only on potato agar. Spore release is observed after rupture of the wall of the sporangium envelope. The oval spores are motile. Pigments are not produced. No growth occurs above 42 °C or below 22 °C; optimal growth occurs at 28–37 °C. Grows at pH 6.0–11.0 and in up to 1.0 % NaCl. Starch hydrolysis, gelatin liquefaction, coagulation of milk,

degradation of casein, nitrate reduction and production of hydrogen sulfide are positive; cellulose decomposition, peptonization of milk and melanin production are negative. L-Arabinose, cellobiose, D-fructose, D-glucose, maltose, sucrose, salicin, L-rhamnose and D-xylose are utilized as sole carbon sources, but *myo*-inositol, D-mannitol, raffinose and lactose are not. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The diagnostic sugars are xylose and arabinose. Mycolic acids are absent. The predominant menaquinone is MK-9(H_6), while minor amounts of MK-9(H_2), and MK-9(H_4) are also detected. The diagnostic phospholipids are phosphatidylethanolamine, hydroxyphosphatidylethanolamine and phosphatidylglycerol. The major fatty acids (>10.0 %) are anteiso-$C_{17:0}$ and iso-$C_{16:0}$, followed by iso-$C_{15:0}$; moderate amounts (5.0–10.0 %) of anteiso-$C_{15:0}$, iso-$C_{17:0}$ and $C_{18:1}\omega9c$ are also present.

The type strain, ATCC 33076^T (=DSM 105064^T=NRRL B-65484^T), was isolated from a soil sample collected in India and identified as a glycolipodepsipeptide producer during a screening programme for discovering novel antibiotics. The G+C content of the genomic DNA of the type strain is 71.4 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Couch JN. *Actinoplanes*, a new genus of the *Actinomycetales*. *J Elisha Mitchell Sci* 1950;66:87–92.
2. Vobis G, Schäfer J, Kämpfer P. Actinoplanes. In: Whitman WB (editor). *Bergey's Manual of Systematics of Archaea and Bacteria*. John Wiley & Sons Inc; 2015. pp. 1–41.
3. Tamura T, Hatano K. Phylogenetic analysis of the genus *Actinoplanes* and transfer of *Actinoplanes minutisporangius* Ruan *et al.* 1986 and '*Actinoplanes aurantiacus*' to *Cryptosporangium minutisporangium* comb. nov. and *Cryptosporangium aurantiacum* sp. nov. *Int J Syst Evol Microbiol* 2001;51:2119–2125.
4. Lechevalier HA, Lechavalier MP. A critical evaluation of the genera of aerobic *Actinomycetes*. In: Prauser H (editor). *The Actinomycetales*. Jena: Gustav Fischer Verlah; 1970. pp. 395–405.
5. Lechevalier MP, de Bievre C, Lechevalier H. Chemotaxonomy of aerobic Actinomycetes: phospholipid composition. *Biochem Syst Ecol* 1977;5:249–260.
6. Goodfellow M, Stanton LJ, Simpson KE, Minnikin DE. Numerical and chemical classification of *Actinoplanes* and some related actinomycetes. *J Gen Microbiol* 1990;136:19–36.
7. Stackebrandt E, Kroppenstedt RM. Union of the genera *Actinoplanes* couch, *Ampullariella* couch, and *Amorphosporangium* couch in a redefined genus *Actinoplanes*. *Syst Appl Microbiol* 1987;9:110–114.
8. Phongsopitanun W, Matsumoto A, Inahashi Y, Kudo T, Mori M *et al.* *Actinoplanes lichenis* sp. nov., isolated from lichen. *Int J Syst Evol Microbiol* 2016;66:468–473.
9. Ngaemthao W, Chunhametha S, Suriyachadkun C. *Actinoplanes subglobosus* sp. nov., isolated from mixed deciduous forest soil. *Int J Syst Evol Microbiol* 2016;66:4850–4855.
10. Cross T. Aquatic actinomycetes: a critical survey of the occurrence, growth and role of actinomycetes in aquatic habitats. *J Appl Bacteriol* 1981;50:397–423.
11. He H, Xing J, Liu C, Li C, Ma Z *et al.* *Actinoplanes rhizophilus* sp. nov., an actinomycete isolated from the rhizosphere of *Sansevieria trifasciata* Prain. *Int J Syst Evol Microbiol* 2015;65:4763–4768.
12. Willoughby LG. A study on acquatic actinomycetes- the allochthonus leaf component. *Nova Hedwigia* 1969;18:45–113.
13. Willoughby LG. Observations on some aquatic Actinomycetes of streams and rivers. *Freshw Biol* 1971;1:23–27.
14. Madden AA, Grassetti A, Soriano JA, Starks PT. Actinomycetes with antimicrobial activity isolated from paper wasp (Hymenoptera: Vespidae: Polistinae) nests. *Environ Entomol* 2013;42:703–710.
15. Tian X, Cao L, Tan H, Han W, Chen M *et al.* Diversity of cultivated and uncultivated actinobacterial endophytes in the stems and roots of rice. *Microb Ecol* 2007;53:700–707.
16. Parenti F, Coronelli C. Members of the genus *Actinoplanes* and their antibiotics. *Annu Rev Microbiol* 1979;33:389–411.
17. Lazzarini A, Cavaletti L, Toppo G, Marinelli F. Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie van Leeuwenhoek* 2001;79:399–405.
18. Marcone GL, Carrano L, Marinelli F, Beltrametti F. Protoplast preparation and reversion to the normal filamentous growth in antibiotic-producing uncommon actinomycetes. *J Antibiot* 2010;63:83–88.
19. Demain AL, Lancini G. Bacterial pharmaceutical products. I. Symbiotic Associations, Biotechnology, Applied Microbiology. In: Dworkin M (editor). *The Prokaryotes. A Handbook on the Biology of Bacteria*, 3rd ed. Berlin: Springer; 2006. pp. 812–833.
20. Cavalleri B, Pagani H, Volpe G, Selva E, Parenti F. A-16686, a new antibiotic from actinoplanes. I. fermentation, isolation and preliminary physico-chemical characteristics. *J Antibiot* 1984;37:309–317.
21. Walker S, Chen L, Hu Y, Rew Y, Shin D *et al.* Chemistry and biology of ramoplanin: a lipoglycodepsipeptide with potent antibiotic activity. *Chem Rev* 2005;105:449–476.
22. Bassères E, Endres BT, Dotson KM, Alam MJ, Garey KW. Novel antibiotics in development to treat *Clostridium difficile* infection. *Curr Opin Gastroenterol* 2017;33:1–7.
23. Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 1966;16:313–340.
24. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA *et al.* *Practical Streptomyces Genetics*. Norwich: John Innes Centre; 2000.
25. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.* Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
26. Larkin MA, Blackshields G, Brown NP, Chenna R, Mcgettigan PA *et al.* CLUSTAL W and CLUSTAL X version 2.0. *Bioinformatics* 2007;23:2947–2948.
27. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
28. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.

29. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
30. **Felsenstein J.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
31. **Kimura M.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
32. **Uchida K, Jang MS, Ohnishi Y, Horinouchi S, Hayakawa M *et al.*** Characterization of *Actinoplanes missouriensis* spore flagella. *Appl Environ Microbiol* 2011;77:2559–2562.
33. **Dalmastri C, Gastaldo L, Marcone GL, Binda E, Congiu T *et al.*** Classification of *Nonomuraea* sp. ATCC 39727, an actinomycete that produces the glycopeptide antibiotic A40926, as *Nonomuraea* gerenzanensis sp. nov. *Int J Syst Evol Microbiol* 2016;66:912–921.
34. **Waksman SA.** Classification, identification, and description of genera and species. *The Actinomycetes*. Baltimore, MD: The Williams & Wilkins Company; 1961. pp. 328–334.
35. **Maerz A, Paul MR.** *A Dictionary of Color*, 2nd ed. New York: McGraw-Hill; 1950.
36. **Goodfellow M, Alderson G, Lacey J.** Numerical taxonomy of *Actinomadura* and related actinomycetes. *J Gen Microbiol* 1979;112:95–111.
37. **Brunati M, Bava A, Marinelli F, Lancini G.** Influence of leucine and valine on ramoplanin production by *Actinoplanes* sp. ATCC 33076. *J Antibiot* 2005;58:473–478.
38. **Staneck JL, Roberts GD.** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 1974;28:226–231.
39. **Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M.** Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* 1980;188:221–233.
40. **Collins MD, Pirouz T, Goodfellow M, Minnikin DE.** Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
41. **Kroppenstedt RM.** Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* 1982;5:2359–2367.
42. **Tindall BJ, Sikorski J, Smibert RM, Krieg NR.** Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM *et al.* (editors). *Methods for General and Molecular Microbiology*, 3rd ed. Washington, DC: American Society for Microbiology; 2007. pp. 330–393.
43. **Kuykendall LD, Roy MA, O'Neill JJ, Devine TE.** Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 1988;38:358–361.
44. **Miller LT.** Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 1982;16:584–586.
45. **Kroppenstedt RM, Stackebrandt E, Goodfellow M.** Taxonomic revision of the actinomycete genera *Actinomadura* and *Microtetraspora*. *Syst Appl Microbiol* 1990;13:148–160.
46. **Cashion P, Holder-Franklin MA, Mccully J, Franklin M.** A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* 1977;81:461–466.
47. **Mesbah M, Whitman WB.** Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine +cytosine of DNA. *J Chromatogr* 1989;479:297–306.
48. **Tamaoka J, Komagata K.** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 1984;25:125–128.
49. **de Ley J, Tijtgat R.** Evaluation of membrane filter methods for DNA–DNA hybridization. *Antonie van Leeuwenhoek* 1970;36:461–474.
50. **Huss VA, Festl H, Schleifer KH.** Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* 1983;4:184–192.
51. **Wayne LG, Moore WEC, Stackebrandt E, Kandler O, Colwell RR *et al.*** Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.