Actinoplanes abujensis sp. nov., isolated from Nigerian arid soil

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A novel actinobacterial strain, A4029^T, isolated from arid soil of Abuja, Nigeria, and provisionally assigned to the genus *Actinoplanes*, was subjected to a polyphasic taxonomic study. 16S rRNA gene sequence similarity studies showed that strain A4029^T belonged to the genus *Actinoplanes*, being most closely related to *Actinoplanes brasiliensis* DSM 43805^T (98.9%) and *Actinoplanes deccanensis* DSM 43806^T (98.0%); similarity to other type strains of the genus *Actinoplanes* ranged from 96.2 to 97.9%. Chemotaxonomic data [major menaquinone MK-9(H₄); major polar lipids phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol; characteristic sugars arabinose and xylose; major fatty acids iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0}, C_{17:1ω9c} and iso-C_{14:0}] confirmed the affiliation of strain A4029^T to the genus *Actinoplanes*. The results of DNA–DNA hybridizations and phylogenetic analysis, together with phenotypic and biochemical test data, allowed strain A4029^T to be differentiated from strains of other *Actinoplanes* species. Therefore, strain A4029^T represents a novel species, for which the name *Actinoplanes abujensis* sp. nov. is proposed, with A4029^T (=DSM 45518^T=NRRL B-24835^T=KCTC 19984^T) as the type strain.

Members of the genus *Actinoplanes*, which was first described by Couch (1950), produce motile spores with spherical, cylindrical, digitate, lobate, bottle or flask-shaped or very irregular sporangia by tufts of polar flagella. Aerial hyphae are scant. *Actinoplanes* strains are also characterized by a number of chemical markers, including the presence of *meso*- and hydroxy-diaminopimelic acid in the whole-cell peptidoglycan, and contain arabinose and xylose as characteristic sugars, MK-9(H₄) as the predominant menaquinone and a genomic DNA G + C content of 67–76 mol% (Goodfellow & Cross, 1984; Kothe, 1987; Vobis, 1989). Phosphatidylethanolamine is the diagnostic phospholipid (Kämpfer *et al.*, 2007). Predominant fatty acids are iso-/anteiso-branched and monounsaturated fatty acids and/or *cis*-9,10-octadecanoic acid. The first detailed phenotypic and chemotypic analysis of the genus was provided by Goodfellow *et al.* (1990), who reported that chemical and numerical taxonomic data supported the integrity of the genus. A comprehensive phylogenetic analysis, based on 16S rRNA gene sequences of members of the genus, was given by Tamura & Hatano (2001). At the time of writing, the genus *Actinoplanes* comprised 28 species with validly published names.

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Actinoplanes abujensis* A4029^T is HQ157185.

A supplementary table and a supplementary figure are available with the online version of this paper.

During a study on the ecological diversity of actinomycetes from different soils, strain A4029^T was isolated on Gauze's medium no. 2 (Gauze *et al.*, 1957) supplemented with cycloheximide (50 µg ml⁻¹), nystatin (50 µg ml⁻¹), nalidixic acid (10 µg ml⁻¹) and novobiocin (10 µg ml⁻¹) incubated at 28 °C for 21 days, from soil samples collected in an arid area, Abuja, Nigeria. The isolate was purified and maintained on glucose-yeast extract-malt extract (GYME; 4 g glucose, 4 g yeast extract, 10 g malt extract, 1 l distilled water, pH 7.2) agar medium and preserved as a suspension of mycelial fragments in glycerol (20%, v/v) at -20 °C.

The morphological and physiological characteristics of strain A4029^T, *Actinoplanes brasiliensis* DSM 43805^T and *Actinoplanes deccanensis* DSM 43806^T were studied together. Morphological characteristics were determined after incubation for 14 days at 28 °C on various media described by Shirling & Gottlieb (1966): yeast extract-malt extract [International *Streptomyces* Project (ISP) 2], oatmeal (ISP 3), inorganic salt-starch (ISP 4), glycerol-asparagine (ISP 5), peptone-yeast extract-iron (ISP 6), tyrosine (ISP 7), modified Bennett's agar (MBA; Jones, 1949), Czapek's agar, trypticase soy agar (TSA) and nutrient agar (NA; Difco). The *National Bureau of Standards Color Name Charts* (Kelly, 1964) was used for determining colour designation and names. Growth at different temperatures was determined on GYME medium incubated at 10, 20, 37 and 45 °C. NaCl tolerance was examined on GYME medium prepared with 1, 2, 3, 4 and 5% (w/v) NaCl. Decomposition of various compounds was examined using the basal medium

recommended by Williams *et al.* (1983). In addition, degradation of RNA (0.5 %, w/v), chitin (0.4 %, w/v) and Tweens 20 and 80 (1 %, w/v) was examined using media and methods described by Goodfellow *et al.* (1979), Hsu & Lockwood (1975) and Nash & Krenz (1991), respectively. Carbon source utilization was tested using carbon source utilization (ISP 9) medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the tested carbon sources and 0.1 % propionic and succinic acids. Nitrogen source utilization was examined using the basal medium recommended by Williams *et al.* (1983) supplemented with a final concentration of 0.1 % of the nitrogen sources tested. Tests in the commercial API 20E and API ZYM systems (bioMérieux) were performed according to the manufacturer's instructions. The ability of strain A4029^T to inhibit the growth of 16 micro-organisms, i.e. Gram-positive and -negative bacteria, yeast and fungi, was observed using an overlay technique described by Williams *et al.* (1983). Spot-inoculated colonies on MBA plates were inverted over 2 ml chloroform for 40 min. Killed colonies were overlaid with 5–7 ml sloppy modified Bennett's broth inoculated with the test organisms. Zones of inhibition were scored as positive results after 24 h at 28 °C.

Cells of strain A4029^T stained Gram-positive. Good growth was observed on most of the tested media. The colour of the substrate mycelium was moderate to deep orange. A strong brown soluble pigment was formed on ISP 7. Cultural characteristics of strain A4029^T in all tested media are presented in Table 1. The physiological properties that differentiated strain A4029^T from *A. brasiliensis* DSM 43805^T and *A. deccanensis* DSM 43806^T are shown in Table 2. *A. brasiliensis* DSM 43805^T and *A. deccanensis* DSM 43806^T could decompose Tween 20, utilize amygdalin, D(–)arabinose, D(–)fructose, D(–)galactose, dextrin, mannitol, melibiose, *myo*-inositol, sucrose and xylose as sole sources of carbon, and utilize L-hydroxyproline and L-methionine as sole sources of nitrogen, but strain A4029^T could not. Other physiological and biochemical features that differentiated strain A4029^T, *A. brasiliensis* DSM 43805^T and *A. deccanensis* DSM 43806^T included NaCl ranges for growth, nitrate reduction, patterns of carbon utilization and antimicrobial activities against *Bacillus pumilus* NRRL BD-142 and *Staphylococcus aureus* ATCC 33862.

Preparation of genomic DNA and PCR amplification of the 16S rRNA gene were carried out according to Chun & Goodfellow (1995). Nearly complete 16S rRNA gene sequences were determined for strain A4029^T (1490 bp) by using an ABI PRISM 3730XL automatic sequencer. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). CLUSTAL W version 1.8 (Thompson *et al.*, 1994) was used to align the sequence of strain A4029^T with those of related taxa retrieved from public databases. Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) tree-making algorithms from the program MEGA

version 3 (Kumar *et al.*, 2004), and the maximum-likelihood method (Felsenstein, 1981) from the PHYLIP suite of programs (Felsenstein, 1993). The evolutionary distance model of Jukes & Cantor (1969) was used to generate evolutionary distance matrices for the neighbour-joining algorithm. Topologies of the resultant trees were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using the CONSENSE and SEQBOOT options from the PHYLIP package.

An almost complete 16S rRNA gene sequence (1490 nt) was determined for strain A4029^T; a 1481 nt fragment was used for phylogenetic analysis and compared against 16S rRNA gene sequences of members of the genus *Actinoplanes*. The phylogenetic tree based on the neighbour-joining algorithm showed that strain A4029^T formed a cluster with most related strain *A. brasiliensis* DSM 43805^T at a high bootstrap resampling value (99.0 %; Fig. 1). Sequence similarity calculations indicated that the closest relatives of strain A4029^T were *A. brasiliensis* DSM 43805^T (98.9 %; 15 nt differences at 1481 locations) and *A. deccanensis* DSM 43806^T (98.0 %; 29 nt differences at 1481 locations); lower sequence similarities (<97.9 %) were found with the type strains of all other members of the genus *Actinoplanes* with validly published names.

DNA–DNA relatedness values between strain A4029^T and *A. brasiliensis* DSM 43805^T were performed by the Identification Service at the Deutsche Sammlung von Mikroorganismen und Zellkulturen Braunschweig, Germany. DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) [incorporating the modifications described by Huß *et al.* (1983)] 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).

The taxonomic integrity of the test strains was supported by DNA relatedness data. Strain A4029^T was determined to have 43.2 % genomic DNA–DNA relatedness (based on a mean of duplicate determinations) with *A. brasiliensis* DSM 43805^T, the phylogenetically most closely related member of the genus *Actinoplanes*, which is clearly well below the 70 % relatedness guideline proposed by Wayne *et al.* (1987) for the delineation of separate species.

Biomass for chemotaxonomic studies was prepared by growing strain A4029^T in GYME broth at 160 r.p.m. for 10 days at 28 °C; cells were harvested by centrifugation, washed in distilled water, re-centrifuged and freeze-dried. Whole-cell hydrolysates were analysed for diaminopimelic acid isomers using TLC (Staneck & Roberts, 1974) and for sugars using the method of Mikami & Ishida (1983). Phospholipids were extracted and analysed by the method of Minnikin *et al.* (1984) as modified by Kroppenstedt & Goodfellow (1991). The predominant isoprenoid quinones were extracted and purified by the method of Tindall

Table 1. Cultural characteristics of strain A4029^T and the most related type strains of the genus *Actinoplanes* on different media
 Strains: 1, A4029^T; 2, *A. brasiliensis* DSM 43805^T; 3, *A. deccanensis* DSM 43806^T. Symbols: + + +, abundant; + +, moderate; +, poor.

Medium	Growth and cultural characteristics	1	2	3
ISP 2	Growth	+ + +	+ + +	+ + +
	Aerial mycelium	None	None	None
	Reverse colour	Deep orange	Deep orange	Pale orange
ISP 3	Soluble pigment	None	None	None
	Growth	+ + +	+ + +	+ +
	Aerial mycelium	None	None	None
ISP 4	Reverse colour	Vivid orange	Brilliant orange	Pale orange–yellow
	Soluble pigment	None	None	None
	Growth	+ +	+ +	+ + +
ISP 5	Aerial mycelium	None	None	None
	Reverse colour	Vivid orange	Vivid orange	Pale orange–yellow
	Soluble pigment	None	None	None
ISP 6	Growth	+ + +	+ + +	+ +
	Aerial mycelium	None	None	None
	Reverse colour	Strong orange	Strong orange	Pale orange
ISP 7	Soluble pigment	None	None	None
	Growth	+ +	+ +	+ +
	Aerial mycelium	None	None	None
GYME	Reverse colour	Brownish orange	Strong brown	Pale orange–yellow/light yellowish
	Soluble pigment	Strong brown	Deep brown	Brown
	Growth	+ + +	+ + +	+ + +
Czapek's agar	Aerial mycelium	None	None	None
	Reverse colour	Deep orange	Deep orange	Pale orange
	Soluble pigment	None	None	None
NA	Growth	+ +	+ +	+ + +
	Aerial mycelium	None	None	None
	Reverse colour	Deep orange–yellow	Vivid orange	Light orange–yellow
TSA	Soluble pigment	None	None	None
	Growth	+ +	+ + +	+ + +
	Aerial mycelium	None	None	None
MBA	Reverse colour	Strong orange	Deep orange	Deep orange
	Soluble pigment	None	None	None
	Growth	+ + +	+ + +	+ + +
	Aerial mycelium	None	None	None
	Reverse colour	Strong orange	Brilliant orange	Pale orange–yellow
	Soluble pigment	None	None	None

(1990a, b) and were determined by reversed-phase HPLC (Wu *et al.*, 1989). In order to quantify whole-cell fatty acid composition, cells were grown in 20 ml trypticase soy broth (TSB) at 28 °C with shaking at 150 r.p.m. After 5 days of incubation, 5 ml of this starter culture was inoculated into 50 ml TSB. The inoculated flask was incubated as before for 5 days. After harvesting by cellulose

membrane filtration (0.45 µm), wet cells (200 mg) were placed in an extraction tube. Cellular fatty acids were extracted, derivatized to their fatty acid methyl esters and separated by the Microbial Identification System (MIDI; Microbial ID), utilizing an Agilent Technologies 6890N GC with a G2614A autosampler and a 6783 injector (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Fatty acid methyl

Table 2. Phenotypic characteristics that differentiate strain A4029^T from the most related members of the genus *Actinoplanes*

Strains: 1, A4029^T; 2, *A. brasiliensis* DSM 43805^T; 3, *A. deccanensis* DSM 43806^T. Symbols: $\pm$, weak; +, positive; –, negative.

Characteristic	1	2	3
Degradation of:			
Tween 20	–	+	+
Tween 80	–	+	–
Growth on sole carbon sources (1.0 %, w/v):			
D(–)Arabinose	–	+	+
D(–)Fructose	–	+	+
D(–)Salicin	–	+	–
D(–)Sorbitol	–	–	+
D(–)Galactose	–	+	+
Dextrin	–	+	+
Lactose	–	–	+
myo-Inositol	–	+	+
Raffinose	–	–	+
Sucrose	–	+	+
Xylose	–	+	+
Growth on sole nitrogen sources (0.1 %, w/v):			
L-Hydroxyproline	–	+	+
L-Methionine	–	+	+
Growth pH	5–9	5–11	5–9
NaCl tolerance (% w/v)	0–3	0–4	0–3
API 20E:			
Citrate	–	–	+
Urease	–	–	+
Gelatin	+	–	+
Mannitol	–	$\pm$	+
Rhamnose	$\pm$	+	+
Melibiose	–	+	+
Amygdalin	–	+	+
Nitrate	+	–	+
API ZYM:			
Esterase	–	+	+
Valine arylamidase	+	–	–
Cystine arylamidase	+	–	–
β -Glucuronidase	–	–	+
α -Mannosidase	–	+	+
Antimicrobial activity against:			
<i>Bacillus pumilus</i> NRRL BD-142	+	–	–
<i>Staphylococcus aureus</i> ATCC 33862	+	–	–

ester peaks were analysed using software version TSBA 50. The DNA G + C content of strains A4029^T was determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

Whole-organism hydrolysates of the organism were rich in *meso*-diaminopimelic acid, xylose and arabinose (wall chemotype II; Lechevalier & Lechevalier, 1970) and phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol

mannosides and different aminophospholipids, phospholipids and glycolipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977) (Supplementary Fig. S1, available in IJSEM Online). The predominant menaquinone of strain A4029^T was MK-9(H₄) (56 %), although substantial amounts of MK-9(H₆), MK-9(H₈) and MK-8(H₄) (18, 6 and 5 %, respectively) were present. The major cellular fatty acids were iso-C_{15:0} (21.1 %), anteiso-C_{15:0} (30.3 %), iso-C_{16:0} (11.7 %), C_{17:1 ω 9c} (7.0 %) and iso-C_{14:0} (7.0 %). Detailed fatty acid profiles are given in Supplementary Table S1 (available in IJSEM Online). The G + C content of the DNA is 70.2 mol%.

It is clear from the genotypic and phenotypic data presented here that strain A4029^T represents a novel species in the genus *Actinoplanes*, for which the name *Actinoplanes abujensis* sp. nov. is proposed.

Description of *Actinoplanes abujensis* sp. nov.

Actinoplanes abujensis (a.bu.jen'sis. N.L. masc. adj. *abujensis* of or belonging to Abuja, Nigeria, source of the organism).

Aerobic, Gram-positive actinomycete. Colony surface is rough; marginal areas are divided into radial and concentric sections. Cells grow well on ISP 2, ISP 3, ISP 5, ISP 7, GYME medium and MBA. Colour of substrate mycelium is orange on several tested media. A strong brown, soluble pigment is formed on ISP 7. Growth occurs at pH 5–9 and 20–37 °C, but not at pH 10.0 or 45 °C. Optimal growth is at 25–30 °C and pH 7.0. Growth is observed in 0–3 % NaCl but not 4 % NaCl (w/v). Positive for acid phosphatase, alkaline phosphatase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, catalase, chymotrypsin, cystine arylamidase, esterase lipase, gelatin, leucine arylamidase, *N*-acetyl- β -glucosaminidase, naphthol-AS-BI-phosphohydrolase, trypsin, valine arylamidase and nitrate reduction; negative for arginine dihydrolase, esterase, lipase, α -fucosidase, α -mannosidase, β -glucuronidase, lysine decarboxylase, ornithine decarboxylase, indole and H₂S production. Aesculin and arbutin are hydrolysed but not allantoin. Casein and starch are degraded, but not adenine, chitin, elastin, guanine, hypoxanthine, Tweens 20 and 80, DNA, RNA, uric acid, xanthine or xylan. D(–)Cellobiose, D(+)glucose, D(–)mannose, maltose and starch are utilized as sole carbon and energy sources, but not adonitol, amygdalin, D(–)arabinose, D(–)fructose, D(–)galactose, D(+)melibiose, D(–)salicin, dextrin, inulin, D(+)sorbitol, L(–)sorbose, lactose, L-glutamate, mannitol, *myo*-inositol, raffinose, sucrose, xylitol or xylose. Utilizes α -isoleucine, D-phenylalanine, glycine, L-alanine, L-arginine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine as sole nitrogen sources, but not L-cysteine, L-histidine, L-hydroxyproline or L-methionine. Antimicrobial activity is shown against *Bacillus pumilus* NRRL BD-142, *Listeria monocytogenes* ATCC 19117, *Staphylococcus aureus* ATCC 33862 and *Staphylococcus aureus* ATCC 25923, but not against *Aspergillus flavus* NRRL 1957^T, *Aspergillus parasiticus* NRRL 465, *Bacillus*

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing relationships between strain A4029^T and members of the genus *Actinoplanes*. Asterisks indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at the nodes indicate the levels of bootstrap support (%); only values above 50 % are cited. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site. The root position of the neighbour-joining tree was obtained using the sequence of *Dactylosporangium aurantiacum* NBRC 12592^T as the outgroup.

cereus NRRL B-3711^T, *Bacillus licheniformis* NRRL B-1001, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Micrococcus luteus* NRRL B-1018, *Pseudomonas aeruginosa* NRRL B-2679, *Proteus vulgaris* NRRL B-123, *Saccharomyces cerevisiae* ATCC 9763 or *Streptomyces murinus* ISP 5091^T. The quinone system consists of MK-9(H₄) (56 %), MK-9(H₆) (18 %) and some minor menaquinones. The polar lipid profile contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. Major fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0}, C_{17:1}ω9c and C_{14:0}.

The type strain, A4029^T (=DSM 45518^T=NRRL B-24835^T=KCTC 19984^T), was isolated from arid soil in Abuja, Nigeria. The G+C content of genomic DNA of the type strain is 70.2 mol%.

Acknowledgements

This research was supported by Ondokuz Mayıs University (OMU), project no. PYO. FEN. 1901.09.003.

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