Nonomuraea jabiensis sp. nov., isolated from arid soil

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A novel actinomycete, strain A4036^T, was isolated from a soil sample collected from the Jabi district in Abuja, Nigeria. The taxonomic position of strain A4036^T was established using a combination of genotypic and phenotypic analyses. The organism formed extensively branched substrate and aerial hyphae that generated spiral chains of spores with warty surfaces. The cell wall contained *meso*-diaminopimelic acid and the cell-wall sugars were glucose, madurose, mannose and ribose. The predominant menaquinone was MK-9(H_4). The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmethylethanolamine, phosphatidylinositol mannoside, hydroxy-phosphatidyl-ethanolamine, hydroxy-phosphatidylmethylethanolamine, two unidentified phospholipids and four unknown glucosamine-containing phospholipids. The major cellular fatty acids were iso-$C_{16:0}$ 2-OH, iso-$C_{16:0}$ and 10-methyl $C_{17:0}$. On the basis of 16S rRNA gene sequence similarity studies, strain A4036^T grouped in the genus *Nonomuraea*, being most closely related to *Nonomuraea angiospora* IFO 13155^T (99.05 %), *Nonomuraea candida* HMC10^T (98.78 %), *Nonomuraea kuesteri* GW 14-1925^T (98.49 %), *Nonomuraea endophytica* YIM 65601^T (98.42 %), *Nonomuraea maheshkhaliensis* 16-5-14^T (98.40 %), *Nonomuraea turkmeniaca* DSM 43926^T (98.38 %), *Nonomuraea helvata* IFO 14681^T (98.29 %), *Nonomuraea rubra* DSM 43768^T (98.10 %) and *Nonomuraea salmonea* DSM 43678^T (98.06 %). Levels of 16S rRNA gene sequence similarity to the type strains of other species of the genus *Nonomuraea* were <98 %. Despite the high 16S rRNA gene sequence similarities, DNA–DNA relatedness values and phenotypic data demonstrated that strain A4036^T was clearly distinguished from all closely related species of the genus *Nonomuraea*. Thus, this isolate is considered to represent a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea jabiensis* sp. nov. is proposed. The type strain is A4036^T (=DSM 45507^T=KCTC 19870^T).

The genus *Nonomuraea*, which was proposed by Zhang *et al.* (1998), belongs to the family *Streptosporangiaceae* and is a member of the suborder *Streptosporangineae* within the order *Actinomycetales*. Members of the genus *Nonomuraea* are aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes which form extensively branched substrate and aerial mycelia. Aerial mycelia differentiate into hooked, spiral or straight chains of spores, which show a folded, irregular, smooth or warty ornamentation (Quintana *et al.*, 2003; Kämpfer *et al.*, 2005). The genus is characterized chemotaxonomically by the presence of *meso*-diaminopimelic acid in the cell wall; madurose as a characteristic sugar in the whole-cell hydrolysates (wall chemotype IIIB *sensu* Lechevalier & Lechevalier, 1970); di-, tetra- and hexahydrogenated menaquinones with nine isoprene units as predominant isoprenologues; major amounts of diphosphatidylglycerol, hydroxylated phosphatidylethanolamine, uncharacterized glycolipids and a glucosamine-containing phospholipid (phospholipid type IV *sensu* Lechevalier & Lechevalier, 1970). The G+C content of the genomic DNA ranges from 64 to 71 mol% (Nonomura & Ohara, 1971; Zhang *et al.*, 1998; Quintana *et al.*, 2003; Hozzein & Goodfellow, 2007; Zhao *et al.*, 2011; Xi *et al.*, 2011). At the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A4036^T is HQ157186.

Five supplementary figures are available with the online version of this paper.

time of writing, the genus *Nonomuraea* contained 30 species with validly published names, which form a distinct phyletic line in the *Streptosporangiaceae* 16S rRNA gene tree (Quintana *et al.*, 2003; Goodfellow *et al.*, 2005; Goodfellow & Quintana, 2006; Wang *et al.*, 2011; Zhao *et al.*, 2011). Strain A4036^T was isolated from a soil sample collected in the Jabi region, Abuja, Nigeria. The aim of this study was to determine the taxonomic position of the isolate using a polyphasic approach.

Strain A4036^T was isolated on Gauze's medium no. 2 [containing (g l^{-1} distilled water): glucose, 10; peptone, 5; tryptone, 3; NaCl, 5; agar, 15; pH 7.2] (Gauze *et al.*, 1957) supplemented with (μg ml^{-1}) cycloheximide (50), nystatin (50), nalidixic acid (10) and novobiocin (10) after incubation for 21 days at 28 °C, following inoculation with a suspension of a soil sample collected in Jabi, Abuja, Nigeria. The organism was maintained on glucose-yeast extract agar slopes (GYEA; Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20 %, v/v) at −20 °C.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product were carried out following Chun & Goodfellow (1995). The almost-complete (1485 bp long) 16S rRNA gene sequence of strain A4036^T was determined using an ABI PRISM 3730 XL automatic sequencer. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence identities were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). The CLUSTAL W version 1.8 program (Thompson *et al.*, 1994) was used to align the sequences of strain A4036^T and 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 MEGA software 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. The 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.

DNA–DNA hybridization analysis was performed between strain A4036^T and its closest neighbours based on 16S rRNA gene sequence similarity, *Nonomuraea angiospora* DSM 43173^T and *Nonomuraea candida* DSM 45086^T. 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 hybridizations were carried out as described by De Ley *et al.* (1970), with the modifications described by Huß *et al.* (1983), using a Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with an *in situ* temperature probe (Varian).

Biomass for chemotaxonomic studies was prepared by growing strain A4036^T in glucose-yeast extract-malt extract broth (Shirling & Gottlieb, 1966) at 160 r.p.m. for 10 days at 28 °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 & Lechevalier (1970) and analysed by TLC (Staneck & Roberts, 1974). Polar lipids were extracted and analysed by the method of Minnikin *et al.* (1984), with the modifications of Kroppenstedt & Goodfellow (2006). Isoprenoid quinones were extracted and purified using the method of Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1982). For the extraction of whole-cell fatty acids, cells were grown in 20 ml of trypticase soy broth (TSB) at 28 °C with shaking at 150 r.p.m. After 5 days of incubation, 5 ml of seed 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 and used to prepare fatty acid methyl esters which were separated by the Microbial Identification System (MIDI; Microbial ID, Inc.), utilizing an Agilent Technologies 6890N gas chromatograph with a G2614A autosampler and a 6783 injector (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Fatty acid methyl ester peaks were analysed using the TSBA database, version 5.0. The DNA G+C content of strain A4036^T was determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

The morphological and physiological characteristics of strain A4036^T, *N. angiospora* DSM 43173^T, *Nonomuraea pusilla* DSM 43357^T, *N. candida* DSM 45086^T, *Nonomuraea spiralis* DSM 43555^T and *Nonomuraea roseoviolacea* subsp. *roseoviolacea* DSM 43144^T were studied together. Cultural characteristics were determined after incubation at 28 and 37 °C for 14 days on various media as described by Shirling & Gottlieb (1966): yeast extract-malt extract agar [International Streptomyces Project (ISP) medium 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), modified Bennett's agar (MBA; Jones, 1949), nutrient agar (NA; Difco) and trypticase soy agar (TSA). The National Bureau of Standards (NBS) Colour Name Charts (Kelly, 1964) were used for determining colour designations and names. Growth tolerance for temperature (4–60 °C) and pH (4–11) were determined on ISP 2 for 7–21 days at 28 and 37 °C. NaCl tolerance (0–5 %, w/v) for growth was observed on ISP 2 at 28 and 37 °C for 14–21 days. Decomposition of various compounds was examined using MBA, as recommended by Goodfellow *et al.* (1979). In addition, degradation of DNA, RNA, chitin and Tweens 20, 40 and 80 (1 %, w/v) was examined using peptone agar (Nash & Krent, 1991). 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 (0.1 % for succinic acid). 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 tested nitrogen sources. Colony morphology and micromorphological properties of isolate A4036^T were determined by examining gold-coated dehydrated specimens of 21-day cultures grown on ISP 2 medium at 28 °C using a JEOL JSM-6060 scanning electron microscope.

The almost-complete 16S rRNA gene sequence (1485 nt) was determined for strain A4036^T; a 1480 nt fragment was used for phylogenetic analysis and compared against 16S rRNA gene sequences of members of the genus *Nonomuraea*. Sequence similarity calculations indicated that the closest relatives of strain A4036^T were *N. angiospora* IFO 13155^T (99.0 %, 14 nt differences at 1407 locations), *N. candida* HMC10^T (98.78 %, 17 nt differences at 1407 locations) and *Nonomuraea kuesteri* GW 14-1925^T (98.49 %, 22 nt differences at 1463 locations). The neighbour-joining tree (Fig. 1), the maximum-parsimony tree and the maximum-likelihood tree (not shown) showed that strain A4036^T formed a clade with *N. angiospora* IFO 13155^T as well as *N. spiralis* IFO 14097^T and *N. pusilla* IFO 14684^T.

Strain A4036^T showed DNA–DNA relatedness values of 12.4 ± 4.2 % to *N. angiospora* DSM 43173^T and 26.0 ± 0.4 % to *N. candida* DSM 45086^T (values are mean of duplicate determinations), clearly well below the 70 % threshold value for the definition of bacterial genomic species (Wayne *et al.*, 1987). Members of the genus *Nonomuraea* have high 16S rRNA gene sequence similarities within the range 97.6−99.8 %, and have low DNA–DNA relatedness values (Fischer *et al.*, 1983; Poschner *et al.*, 1985; Tamura *et al.*, 2000; Kämpfer *et al.*, 2005, 2010; Ara *et al.*, 2007; Zhao *et al.*, 2011). Based on these findings, DNA–DNA hybridizations between strain A4036^T and its phylogenetic neighbours *N. spiralis* DSM 43555^T and *N. pusilla* DSM 43357^T were not performed (<98.0 % 16S rRNA gene sequence similarity).

Strain A4036^T contained *meso*-diaminopimelic acid (cell wall type III; Lechevalier & Lechevalier, 1970) as the cell-wall diamino acid and the whole-cell sugars were glucose, ribose (major components), mannose and madurose (Type B; madurose was the diagnostic sugar). The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmethylethanolamine, phosphatidylinositol mannoside, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylmethylethanolamine, two unidentified phosphplipids and four unidentified glucosamine-containing phospholipids (Figs S1–3, available in IJSEM Online). The predominant menaquinone of strain A4036^T was MK-9(H_4) (58.0 %); MK-9(H_2) (21.0 %), MK-9(H_0) (13.0 %) and MK-9(H_6) (5.0 %) were also detected (Fig. S4). The major cellular fatty acids were iso-$C_{16:0}$ 2-OH (29.48 %), iso-$C_{16:0}$ (20.14 %) and 10-methyl $C_{17:0}$ (21.21 %); minor amounts of iso-$C_{16:1}$ G (8.14 %), iso-$C_{15:0}$ (6.6 %), *cis*9-$C_{17:1}$ (5.11 %), $C_{15:0}$ (2.53 %), $C_{15:0}$ 2-OH (2.06 %), 10-methyl $C_{16:0}$ (1.6 %) and 10-methyl iso-$C_{17:0}$ (3.01 %) were also present. The G+C content of the DNA was 69.6 mol%.

The morphological characteristics of strain A4036^T were consistent with those of members of the genus *Nonomuraea*. Strain A4036^T showed good growth on ISP 2, NA, TSA and MBA, poor growth on ISP 3, ISP 4 and ISP 7, and no growth on ISP 5 and ISP 6. White aerial hyphae were formed on MBA. The colour of the substrate mycelium was brownish orange. Diffusible pigments were not produced. Melanoid pigments were not produced on ISP 7 medium. Strain A4036^T formed extensively branched substrate and aerial hyphae that often formed spiral chains

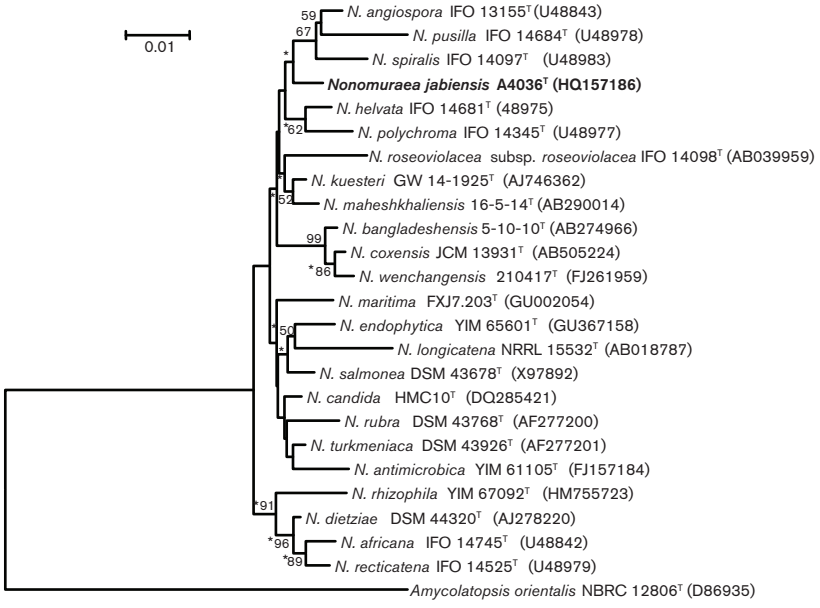

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16 rRNA gene sequences (1480 nt) showing the position of strain A4036^T amongst its phylogenetic neighbours. *Amycolatopsis orientalis* NBRC 12806^T was used as an outgroup. Asterisks indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Numbers at nodes indicate the levels of bootstrap support (%); only values ⩾50 % are shown. Bar, 0.01 substitutions per site.

Table 1. Cultural characteristics on different media of strain A4036^T and the most closely related type strains of species of the genus *Nonomuraea*

Strains: 1, A4036^T; 2, *N. angiospora* DSM 43173^T; 3, *N. candida* DSM 45086^T; 4, *N. spiralis* DSM 43555^T; 5, *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T; 6, *N. pusilla* DSM 43357^T. G, growth; AM, aerial mycelium; RC, reverse colour; SP, soluble pigment; +++, abundant; ++, moderate; +, poor.

Medium		1	2	3	4	5	6
ISP 2	G	+++	+++	+++	+++	+++	+++
	AM	None	White	Yellowish white	White	Pale orange yellow	White
	RC	Deep brown	Deep orange yellow	Deep yellowish brown	Light orange yellow	Reddish black	Strong brown
	SP	None	None	None	None	Deep reddish orange	None
ISP 3	G	+	+++	No growth	+++	+++	+++
	AM	None	Pale yellow		Yellowish white	Vivid yellowish pink	White
	RC	Brownish orange	Brownish orange		Yellowish white	Dark reddish orange	Dark orange yellow
	SP	None	None		None	Strong reddish orange	None
ISP 4	G	+	++	+	+++	++	No growth
	AM	None	None	White	Yellowish white	White	
	RC	Brownish orange	Strong orange yellow	Moderate yellowish brown	Yellowish white	Moderate red	
	SP	None	None	None	None	None	
ISP 5	G	No growth	++	++	+++	+	No growth
	AM		None	None	White	None	
	RC		Moderate orange yellow	Moderate orange yellow	Moderate orange yellow	Moderate reddish orange	
	SP		None	None	None	None	
ISP 6	G	No growth	+++	No growth	+++	++	+++
	AM		None		None	None	None
	RC		Brownish orange		Moderate orange yellow	Dark reddish orange	Strong brown
	SP		None		None	Greyish reddish orange	None
ISP 7	G	+	++	+	+++	++	No growth
	AM	None	White	Pale orange yellow	White	White	
	RC	Brownish orange	Light orange yellow	Moderate yellow	Moderate orange yellow	Moderate orange yellow	
	SP	None	None	None	None	None	
Nutrient agar	G	+++	++	+++	++	+++	+++
	AM	None	None	Brownish pink	None	None	None
	RC	Deep orange	Moderate orange yellow	Dark olive brown	Orange yellow	Vivid red	Deep reddish brown
	SP	None	None	Greyish yellow	None	Greyish red	Dark pink
Trypticase soy agar (TSA)	G	+++	+++	+++	++	+++	+++
	AM	None	None	None	None	None	None
	RC	Deep orange	Vivid orange yellow	Deep yellowish brown	Light yellow	Strong reddish orange	Strong brown
	SP	None	None	None	None	Dark orange yellow	Deep orange yellow
Modified Bennett's agar (MBA)	G	+++	+++	+++	+++	+++	+++
	AM	White	Yellowish white	None	None	None	None
	RC	Deep orange	Moderate orange yellow	Moderate olive	Moderate orange yellow	Strong brown	Deep brown
	SP	None	None	Deep greenish yellow	None	Moderate reddish orange	Light orange

of spores with warty surfaces (Fig. S5). Cultural characteristics of strain A4036^T and related type strains on all tested media are presented in Table 1. The physiological properties that distinguish strain A4036^T from closely related species of the genus *Nonomuraea* are listed in Table 2.

On the basis of the phenotypic and genotypic data, strain A4036^T represents a novel species within the genus *Nonomuraea*, for which the name *Nonomuraea jabiensis* sp. nov. is proposed.

Table 2. Phenotypic properties that differentiate strain A4036^T from the most closely related species of the genus *Nonomuraea*

Strains: 1, A4036^T; 2, *N. angiospora* DSM 43173^T; 3, *N. candida* DSM 45086^T; 4, *N. spiralis* DSM 43555^T; 5, *N. roseoviolacea* subsp. *roseoviolacea* DSM 43144^T; 6, *N. pusilla* DSM 43357^T. +, Positive; −, negative.

Characteristic	1	2	3	4	5	6
Biochemical tests						
Allantoin hydrolysis	−	−	−	−	+	+
Nitrate reduction	+	−	−	+	+	+
Degradation of:						
Adenine	+	−	+	−	−	−
Casein	+	−	−	−	−	−
Elastin	+	+	−	−	−	−
Gelatin	+	+	−	+	−	−
Hypoxanthine	+	+	−	−	+	−
Starch	−	+	−	−	−	−
Testosterone	+	+	−	+	+	−
Tween 20	+	+	+	−	+	+
Tween 40	+	+	+	−	+	+
Tween 80	+	+	+	−	−	−
Xylan	+	+	−	−	−	−
Growth on sole carbon sources (1.0 %, w/v)						
Adonitol	+	+	−	−	+	+
Arbutin	−	+	+	−	+	+
Cellobiose	−	−	+	−	+	+
D-Fructose	+	+	+	−	+	−
D-Sorbitol	−	−	+	−	−	−
D-Galactose	−	+	+	−	−	−
D-Mannose	+	+	+	+	−	−
D-Melezitose	+	−	−	+	+	−
D-Mannitol	+	+	+	−	+	−
Dextrin	+	+	+	−	+	+
Inulin	+	+	+	−	+	−
L-Sorbose	−	−	+	−	−	−
L-Glutamate	−	−	+	−	+	+
Maltose	+	+	+	−	+	−
myo-Inositol						
Sucrose	+	+	+	+	+	−
Xylose	+	+	+	+	+	−
Succinic acid (0.1 %)	+	+	+	−	+	−
Growth on sole nitrogen sources (0.1 %, w/v)						
D-Phenylalanine	+	+	+	+	−	+
L-Valine	+	+	−	−	−	−
Growth with/at:						
3 % (w/v) NaCl	−	+	+	−	+	+
pH 11.0	−	−	+	−	−	−

Description of *Nonomuraea jabiensis* sp. nov.

Nonomuraea jabiensis (ja.bi.en′sis. N.L. fem. adj. *jabiensis* of or belonging to Jabi, Abuja, Nigeria, the source of the type strain).

Aerobic, Gram-positive, non-motile actinomycete which forms extensively branched, brownish orange substrate mycelia that bear white aerial hyphae on MBA. Aerial mycelia bear spiral chains of spores with warty surfaces. Diffusible pigments are not produced. Melanoid pigment is not produced on ISP 6 or ISP 7 agars. Growth occurs at pH 5.0–10.0, and at 20–37 °C, but not at pH 4.0 or pH 11.0, or at 10 or 45 °C. Optimal growth occurs at 28 °C and pH 7.0. Growth is observed in the presence of 0–2 % (w/v) NaCl. Positive result in tests for arbutin, aesculin and urea hydrolysis and nitrate reduction, but negative result for allantoin hydrolysis. Adenine, casein, elastin, gelatin, hypoxanthine, testosterone, Tweens 20, 40 and 80, and xylan are degraded, but not chitin, DNA, guanine, RNA or starch. Adonitol, D-fructose, D-mannose, D-melezitose, melibiose, D-mannitol, dextrin, inulin, L-rhamnose, lactose, maltose, sucrose, xylose and succinic acid are utilized as sole carbon sources, but L-arabinose, cellobiose, dextran, L-glutamate, D-sorbitol, D-galactose, L-sorbose, *myo*-inositol and xylitol are not. Utilizes α-isoleucine, D-phenylalanine, L-alanine, L-arginine, L-cysteine, L-leucine, L-histidine, L-hydroxyproline, L-phenylalanine, L-proline, L-methionine, L-serine, L-threonine, L-tyrosine and L-valine, but not glycine, as sole nitrogen sources. The predominant menaquinone is MK-9(H_4), with minor amounts of MK-9(H_2), MK-9(H_0) and MK-9(H_6) also detected. The polar lipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmethylethanolamine, phosphatidylinositol mannoside, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylmethylethanolamine, two unidentified phospholipids and four unknown glucosamine-containing phospholipids. Major fatty acids are iso-$C_{16:0}$ 2-OH, iso-$C_{16:0}$ and 10-methyl $C_{17:0}$.

The type strain, A4036^T (=DSM 45507^T=KCTC 19870^T), was isolated from arid soil collected from the Jabi region in Abuja, Nigeria. The G+C content of the genomic DNA of the type strain is 69.6 mol%.

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