Nocardiopsis arvandica sp. nov., isolated from sandy soil

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An actinomycete strain, HM7^T, was isolated from sandy soil from the banks of the Arvand River, Khoramshahr, Iran. In a study conducted using a polyphasic taxonomic approach, it was found that this strain represented a novel species in the genus *Nocardiopsis*. Strain HM7^T had long, branched substrate mycelia and the aerial mycelium completely fragmented to long chains of rod-shaped spores. The cell wall of strain HM7^T contained *meso*-diaminopimelic acid, galactose and ribose. The predominant phospholipids were phosphatidylglycerol, phosphatidylethanolamine diphosphatidylglycerol, and phosphatidylcholine. The main menaquinones were MK-10(H₂), MK-10(H₄), MK-10(H₀) and MK-9(H₂). Strain HM7^T showed the highest degree of 16S rRNA gene sequence similarity with *Nocardiopsis sinuspersici* UTM 00102^T (99.8%) and *Nocardiopsis quinghaiensis* YIM 28A4^T (99.3%), but differed significantly from these type strains based on DNA–DNA hybridization data (37.6 and 38.9% relatedness, respectively). Physiological, biochemical and phenotypic characteristics allowed strain HM7^T to be differentiated from related species. On the basis of results from phenotypic and phylogenetic analyses, strain HM7^T is considered to represent a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis arvandica* sp. nov. is proposed; the type strain of this species is HM7^T (=UTMC 00103^T=DSM 45278^T=CCUG 58831^T).

Members of the genus *Nocardiopsis* are mesophilic, aerobic actinomycetes that produce arthrospores on aerial mycelia. At the time of writing, the genus *Nocardiopsis* harboured 29 recognized species. The natural habitat of most recognized members of the genus is soil (Kroppenstedt & Evtushenko, 2002). Species of the genus *Nocardiopsis* have also been found in an Antarctic glacier, in marine sediments, in the actinorhizal plant rhizosphere, in the gastrointestinal tract of animals, as endophytes of yam bean, in salterns and in clinical material. Nearly all recognized species of the genus *Nocardiopsis* have, up until now, been found at sites of moderate temperatures. Thermotolerant strains, which are able to grow at temperatures of up to 42 °C or even at 50 °C, have their optimal growth temperature at 28 °C (Evtushenko *et al.*, 2000) or, in the case of *Nocardiopsis kunsanensis*, at 37 °C (Chun *et al.*, 2000). Members of this genus frequently have been isolated from areas with high salt concentrations, such as *Nocardiopsis lucentensis* from a

salt marsh soil, a *Nocardiopsis halotolerans* strain from the same environment, *Nocardiopsis halophila* from a saline soil and *N. kunsanensis* from a saltern. Mildly alkaline conditions (pH 8) are best for optimal growth of most strains of this genus. For some species, i.e. *Nocardiopsis prasina* and *Nocardiopsis dassonvillei* subsp. *albirubida*, growth is enhanced at even higher pH values. Generally, many of the species of the genus *Nocardiopsis* prefer moderately alkaline conditions (pH 8.5) (Kroppenstedt, 1992) and some grow better on media supplemented with sodium chloride. Therefore, *Nocardiopsis* strains are frequently isolated from alkaline soils with high salt concentrations (Mikami *et al.*, 1982; Yassin *et al.*, 1993).

In this paper, the taxonomic characterization and classification of strain HM7^T, an isolate from sandy soil, is reported and a novel species in the genus *Nocardiopsis* is proposed.

Strain HM7^T was isolated from a soil sample collected from sandy soil (pH 8.7, salinity 2.6%) taken at a depth of 10 cm on the right bank of the Arvand River in Khoramshahr city, Khuzestan province, Iran. The soil

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UTM 00103^T is EU410477.

Supplementary figures are available with the online version of this paper.

Table 1. Cultural characteristics of strain HM7^T

Colours were taken from ISCC–NBS colour charts (Kelly, 1964).

Medium*	Growth	Aerial mycelium	Substrate mycelium
Yeast extract-malt extract (ISP 2)	Good	Light brown	Light brown
Oatmeal agar (ISP 3)	Relatively poor	White	Pale yellow
Inorganic salts-starch agar (ISP 4)	Poor	Light yellow	Light yellow
Glycerol-asparagine agar (ISP 5)	Very poor	Pale brown	Pale brown

*ISP, International *Streptomyces* Project (Shirling & Gottlieb, 1966).

sample was dried (Nolan & Cross, 1988), oven-dried at 120 °C for 10 min (Nonomura & Ohara, 1971) and then irradiated at 257 nm for 10 min (Galatenko & Terekhova, 1990). Soil samples were then treated with a phosphate buffer-soil extract solution (Hayakawa *et al.*, 2000) and chlorhexidine gluconate (Hayakawa *et al.*, 1991). Strains were isolated by a dilution plating method on AV agar (Nonomura & Ohara, 1969) supplemented with cycloheximide (100 µg ml⁻¹) after 21 days incubation at 28 °C. Strains were maintained on ISP 2 agar slants at 4 °C and as 20% (w/v) glycerol suspensions at -20 °C.

Cultural characteristics as well as soluble pigments were studied on ISP media as described by Shirling & Gottlieb (1966). The colours of both substrate and aerial mycelia were determined by comparison with chips from ISCC–NBS colour charts (Kelly, 1964) and soluble pigment production was determined.

The novel isolate was cultivated on ISP 2 agar for 14 days at 28 °C and cells were used for microscopic observations of sporophores, spore chains and the spore surface by using a light microscope with a coverslip technique (Kawato & Shinobu, 1959) and a scanning electron microscope (CEM902A; Zeiss).

The colour of the aerial mycelium varied from white to pale brown on ISP media. The substrate mycelium was light yellow to pale brown. No soluble pigments were released on any medium (Table 1). Vegetative hyphae of strain HM7^T were long, well-developed and fragmented. Long spore chains were borne on the aerial hyphae. Strain HM7^T showed the typical microscopic appearance of most species of the genus *Nocardiopsis* (Meyer, 1994). Spores were rod-shaped (0.4 × 1.5 µm) and smooth-surfaced (see Supplementary Fig. S1 in IJSEM Online).

Carbon source utilization was tested on ISP 9 as a basal medium supplemented with a final concentration of 1% carbon source and 0.1% organic acid source (Shirling & Gottlieb, 1966). Utilization of nitrogen sources, growth on other organic compounds, degradation activity and enzymic activity were determined as described by Williams *et al.* (1983).

The temperature range for growth of strain HM7^T was 15–45 °C, with optimum growth at 25–30 °C. The strain

showed good growth at NaCl concentrations up to 17.5%, but was not able to grow in the presence of 20% NaCl. Strain HM7^T could grow well at pH 5–12, but could not grow at pH 4 or 13.

For chemotaxonomic and molecular studies, strain HM7^T was cultured in GYM broth medium (Shirling & Gottlieb, 1966) and incubated at 28 °C for 6 days at 200 r.p.m. Mycelia were harvested by centrifugation and washed twice with deionized water. Amino acid and sugar analyses of whole-cell hydrolysates were performed as described by Stanek & Roberts (1974). Menaquinones and phospholipids were extracted by the integrated method of Minnikin

Table 2. Cellular fatty acid composition (%) of strain HM7^T and closely related members of the genus *Nocardiopsis*

Strains: 1, HM7^T; 2, *N. sinuspersici* DSM 45277^T; 3, *N. quinghaiensis* DSM 44739^T; 4, *N. aegyptia* DSM 44442^T; 5, *N. halotolerans* DSM 44410^T. –, Not detected; ND, not reported.

Fatty acids	1	2	3	4	5
iso-C _{12:0}	0.2	ND	ND	ND	ND
iso-C _{13:0}	0.5	ND	ND	ND	ND
anteiso-C _{13:0}	0.8	ND	ND	ND	ND
iso-C _{14:0}	3.1	2.5	5.6	12.1	2.4
iso-C _{15:0}	1.5	0.7	2.6	1.7	3.4
anteiso-C _{15:0}	13.4	6.7	14.6	6.5	16.7
iso-C _{16:0}	22.8	31.1	30.0	19.1	23.3
C _{16:0}	2.2	0.6	3.7	4.2	3.2
C _{16:1}	0.3	0.3	ND	1.10	1.0
10-methyl-C _{16:0}	0.6	0.7	1.8	1.5	–
C _{16:1}	1.7	0.3	–	1.1	1.3
iso-C _{17:0}	2.4	2.1	2.2	5.1	4.2
anteiso-C _{17:0}	19.2	19.2	10.0	17.9	18.7
C _{17:0}	4.6	2.0	2.8	1.3	3.1
C _{17:1}	2.4	1.6	2.7	1.7	6.8
anteiso-C _{17:1}	0.4	0.4	–	1.4	–
10-methyl-C _{17:0}	3.3	5.8	2.8	2.6	6.6
iso-C _{18:0}	2.7	4.2	1.4	3.2	1.3
C _{18:0}	8.8	6.0	4.5	7.3	3.5
C _{18:1}	3.3	3.3	4.6	7.8	8.5
10-methyl-C _{18:0}	5.5	8.8	4.5	12.2	1.3
anteiso-C _{19:0}	0.8	ND	ND	ND	ND

et al. (1984). The resultant phospholipids were separated and identified as described by Minnikin *et al.* (1984). The purified menaquinones were analysed by using HPLC (Kroppenstedt, 1982, 1985). Cellular fatty acid methyl esters were prepared and analysed according to the method of Sasser (1990).

Strain HM7^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid in whole-cell hydrolysates. Galactose and ribose were the only sugars found in the hydrolysates [cell wall type III, according to Lechevalier & Lechevalier (1980)]. The polar lipids comprised the diagnostic phospholipids phosphatidylcholine, diphosphatidylglycerol and phosphatidylethanolamine, as well as the non-diagnostic phosphatidylinositol, phosphatidylglycerol and unknown glycolipids (see Supplementary Fig. S2 in IJSEM Online). The phospholipid pattern was type III according to Lechevalier *et al.* (1977). The phospholipid patterns matched those found in other species of the *Nocardiopsis* (Kroppenstedt & Evtushenko, 2002). The

menaquinones of strain HM7^T were mainly of the MK-10 type: MK-10(H₂), 35 %; MK-10(H₄), 22 %; MK-10(H₀), 19 %; MK-9(H₂), 18 %; and MK-9(H₀), 5 %. The fatty acid spectrum contained mainly saturated branched-chain acids: iso-C_{16:0} (22.8 %), anteiso-C_{17:0} (19.2 %), anteiso-C_{15:0} (13.4 %), C_{18:0} (8.8 %), 10-methyl-C_{18:0} (5.5 %), 10-methyl-C_{17:0} (3.3 %), *cis*-9-C_{18:1} (3.3 %), iso-C_{14:0} (3.1 %) and iso-C_{18:0} (2.7 %) (Table 2). The fatty content was characteristic for members of the genus *Nocardiopsis* (Kroppenstedt, 1985, 1992). All chemotaxonomic properties of strain HM7^T were consistent with its classification within the genus *Nocardiopsis* (Kroppenstedt & Evtushenko, 2002).

Chromosomal DNA was prepared using a DNA extraction kit (JetFlex). The 16S rRNA gene was enzymically amplified using the oligonucleotide primers 10-30F (5'-GAGTTT-GATCCTGGCTCA-3') and 1500R (5'-AGAAAGGAGGT-GATCCAGCC-3'), as described by Rainey *et al.* (1996). PCR products were purified with a PCR product purification kit (Qiagen). 16S rRNA gene sequences of members of the

Fig. 1. Phylogenetic tree highlighting the position of strain HM7^T relative to all type strains of the family *Nocardiopsaceae* inferred from 1344 aligned characters (total alignment length was 1646 bp) of the 16S rRNA gene sequence under the maximum-likelihood criterion. Rooting was done with the type strains of the genera *Haloactinospora*, *Marinactinospora* and *Thermobifida*. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1000 bootstrap replicates; only values greater than 60 % are shown.

Table 3. Characteristics that differentiate strain HM7^T from the most closely related *Nocardiopsis* species

Strains: 1, HM7^T; 2, *N. sinuspersici* DSM 45277^T; 3, *N. quinghaiensis* DSM 44739^T; 4, *N. aegyptia* DSM 44442^T; 5, *N. halotolerans* DSM 44410^T. +, Positive; –, negative; –(+), weakly positive; ND, no data; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

Characteristic	1	2	3	4	5
Utilization of:					
D-Galactose	+	+	–	+	+
L-Arabinose	–	–	+	+	–
Melibiose	+	+	–	+	+
Adonitol	–	–	–	ND	–
Glycerol	+	+	+	+	+
Inositol	–	–	+	+	–
Sucrose	–	+	+	+	+
Ribose	+	–	–	–	–
Lactose	–	+	–	– (+)	–
Mannitol	–	+	+	+	–
D-Mannose	–	+	+	+	+
L-Rhamnose	–	+	–	+	–
D-Xylose	–	+	–	+	–
Raffinose	–	–	–	–	–
Gluconate	+	–	–	–	–
Propionate	–	+	–	+	–
Adenine	+	+	+	–	–
Allantoin	+	–	+	+	–
Growth at/in:					
10 °C	–	–	+	+	+
40 °C	+	–	–	–	–
5 % NaCl	+	+	+	–	+
10 % NaCl	+	+	+	–	+
15 % NaCl	+	+	–	–	+
20 % NaCl	–	–	–	–	–
pH 12	+	+	–	+	–
Major menaquinones*	10/2, 10/4, 10/0, 9/2, 9/0	10/0, 9/0, 10/2, 9/2, 10/4	10, 10/2, 11/2, 11	10/6, 10/8	10/0, 10/2
Polar lipids	PI, PG, PC, PE, DPG	PG, PE, PC, PI	PC, PG, DPG	PC, PME, PI, PG, DPG	PC, PI, PG, PME, DPG

*Nomenclature, e.g. 10/4, MK-10(H₄).

family *Nocardiopsaceae* were aligned with POA v.2 (Lee *et al.*, 2002) in progressive mode in conjunction with BLASTN (Altschul *et al.*, 1990) and ambiguous regions were subsequently removed using Gblocks (Castresana, 2000) under default settings. Maximum-likelihood bootstrapping and a subsequent search for the best-known tree were conducted using RAXML v.7.04 (Stamatakis *et al.*, 2008) with 1000 replicates of the novel fast bootstrap algorithm and GTR + GAMMA as the substitution model, approximated with GTR + CAT during the tree search (command-line switches –f b –m GTRMIX). The tree was rooted with the *Haloactinospira*–*Marinactinospira*–*Thermobifida* clade included in the sample and is shown in Fig. 1. The DNA G + C content of strain HM7^T was determined by HPLC (Mesbah *et al.* 1989; Tamaoka & Komagata, 1984).

DNA for hybridization experiments was prepared using a French pressure cell (Thermo Spectronic) and was 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) with the modifications of 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 an *in situ* temperature probe (Varian).

According to the aligned, almost complete 16S rRNA gene sequences, strain HM7^T was most similar to members of the genus *Nocardiopsis*, with the highest similarity to *Nocardiopsis sinuspersici* DSM 45277^T (Hamedí *et al.*, 2010) (99.8 %) and *Nocardiopsis quinghaiensis* YIM 28A4^T (Chen *et al.*, 2008) (99.3 %); similar values were found for other species of the genus *Nocardiopsis*. When the phylogenetic position of strain HM7^T was investigated with all recognized species of the genus *Nocardiopsis*, the strain formed a monophyletic clade with *N. sinuspersici* DSM

45277^T and *N. quinghaiensis* YIM 28A4^T with 99 % bootstrap support. Fig. 1 shows a phylogenetic dendrogram displaying the relatedness of strain HM7^T to type strains of the genus *Nocardiopsis*.

DNA–DNA hybridization studies showed that the DNA–DNA relatedness between strain HM7^T and *N. sinuspersici* DSM 45277^T was 37.6 % (± 1.1), whereas *N. quinghaiensis* DSM 44739^T was related to these two strains by 39.6 % (± 0.2) (*N. sinuspersici* DSM 45277^T) and 38.9 % (± 2.1) (HM7^T). The observed levels of DNA–DNA relatedness demonstrated the genomic distinctiveness of strain HM7^T when compared with *N. sinuspersici* DSM 45277^T and *N. quinghaiensis* DSM 44739^T.

Strain HM7^T differed chemotaxonomically from its sister taxa *N. sinuspersici* DSM 45277^T and *N. quinghaiensis* DSM 44739^T, thus enabling it to be clearly differentiated. These strains showed different fatty acid types (Table 2), predominant menaquinones and phospholipid patterns (Table 3). Strain HM7^T could be differentiated from *N. sinuspersici* DSM 45277^T by means of phenotypic properties such as the consumption of a broad range of compounds including sucrose, ribose, lactose, mannitol, D-mannose, L-rhamnose, D-xylose, gluconate, propionate and allantoin. It could also be differentiated from *N. quinghaiensis* DSM 44739^T by the consumption of D-galactose, L-arabinose, melibiose, inositol, sucrose, ribose, mannitol, D-mannose and gluconate (Table 3). Based on the above phenotypic and genotypic results, it is concluded that isolate HM7^T merits species status within the genus *Nocardiopsis* and the name *Nocardiopsis arvandica* sp. nov. is proposed for this isolate, with HM7^T (=UTMC 00103^T=DSM 45278^T=CCUG 58831^T) as the type strain.

Description of *Nocardiopsis arvandica* sp. nov.

Nocardiopsis arvandica (ar.van'di.ca. N.L. fem. adj. *arvandica* of or belonging to Arvand, referring to the geographical origin of the type strain, the Arvand River).

Aerobic, Gram-positive, non-acid-fast actinomycete that forms an extensively branched substrate mycelium and abundant aerial mycelium. Hyphae of the aerial mycelium are straight to flexuous. In older cultures, hyphae of aerial mycelium differentiate into rod-shaped, smooth-surfaced spores. Soluble pigments, including melanin, are not produced. A light brown substrate mycelium and a light brown aerial mycelium are produced on yeast extract-malt extract agar. Optimal growth occurs at 25–30 °C. Good growth occurs in media containing 0–15 % NaCl. Grows at pH 6–11. D-Galactose, melibiose, glycerol, ribose and gluconate are used as sole carbon sources, but not L-arabinose, adonitol, inositol, sucrose, lactose, mannitol, D-mannose, D-xylose, L-rhamnose, raffinose or propionate. Utilizes L-alanine, L-asparagine, L-proline, L-serine, L-arginine, L-tryptophan, cysteine, histidine, phenylalanine, lysine, ornithine and L-valine as the sole nitrogen sources. Decomposes allantoin and adenine. Whole cell hydrolysates contain *meso*-diaminopimelic acid, but no diagnostic sugars.

Polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol. Menaquinones include MK-10(H₂), MK-10(H₄), MK-10(H₀) and MK-9(H₂). The predominant fatty acids in whole-cell methanolysates are iso-C_{16:0} and anteiso-C_{17:0}.

The type strain is HM7^T (=UTMC 00103^T=DSM 45278^T=CCUG 58831^T), isolated from a sandy soil from the banks of the Arvand River, Khoramshahr, Khuzestan, Iran. The DNA G+C content of strain HM7^T is 71.5 mol%.

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