Nocardiopsis alkaliphila sp. nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt

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An alkaliphilic actinomycete strain, designated YIM 80379T, was isolated from a soil sample collected from the eastern desert of Egypt and subjected to polyphasic taxonomy. The strain produced substrate and aerial mycelia on different media, with an optimum pH for growth of 9.5–10 and scarce or no growth at pH 7. Strain YIM 80379T contained meso-diaminopimelic acid, no diagnostic sugars, type III phosopholipids and MK-10(H6) and MK-10(H8) as the predominant menaquinones. All of these characters assign isolate YIM 80379T consistently to the genus Nocardiopsis. This was confirmed by 16S rDNA analysis. It can be differentiated from all Nocardiopsis species with validly published names by phenotypic characteristics, phylogenetic analysis and DNA–DNA hybridization results. On the basis of polyphasic evidence, a novel species, Nocardiopsis alkaliphila sp. nov., is proposed. The type strain of the species is YIM 80379T (= CECTCC AA001031T = DSM 44657T).

Although alkaliphilic bacteria have been studied extensively for a long time, work on alkaliphilic actinomycetes is very rare. This is clear from the fact that very few articles about alkaliphilic actinomycetes have been published previously (Miyashita et al., 1984; Groth et al., 1997; Duckworth et al., 1998; Kroppenstedt & Evtushenko, 2002). It is therefore important to pay more attention to this group of extreme actinomycetes, as a possible way to discover novel taxa and, consequently, new secondary metabolites. It was reported that most of the alkaliphilic actinomycetes that have been isolated belong to the genus Nocardiopsis (Mikami et al., 1982, 1986; Kroppenstedt & Evtushenko, 2002); most Nocardiopsis species are alkaliphilic, as their growth optimum is above pH 8–9 and the genus Nocardiopsis contained 16 species with validly published names at the time of writing (Meyer, 1976; Grund & Kroppenstedt, 1990; Yassin et al., 1993, 1997; Al-Tai & Ruan, 1994; Chum et al., 2000; Evtushenko et al., 2000; Peltola et al., 2001; Al-Zarban et al., 2002; Kämpfer et al., 2002; Schippers et al., 2002; Li et al., 2003). In this study, a novel alkaliphilic actinomycete was identified by a polyphasic approach and was found to be a novel species of the genus Nocardiopsis. The name Nocardiopsis alkaliphila sp. nov. is proposed.

Strain YIM 80379T was isolated from a soil sample collected from the eastern desert of Egypt by using medium A, which was recommended by Sato et al. (1983) for the isolation of alkaliphilic and alkaline-resistant micro-organisms. This medium contained (g l–1): glucose, 10–0; peptone, 5–0; yeast extract, 5–0; KH2PO4, 1·0; MgSO4·7H2O, 0·2; Na2CO3, 10–0; and agar, 15–0. Sodium carbonate was sterilized separately and then added to the medium. The pH of the medium was 10·0–10·5. NaHCO3/Na2CO3 buffer was used to adjust the pH. After incubation at 28°C for 14 days, a visible colony (designated YIM 80379T) was picked and subcultured until purification. A preliminary test was carried out to confirm its requirement for alkalinity; it was unable to grow below pH 7·0. The strain was maintained in 20% glycerol and kept at –20°C.

The isolate was cultivated on medium A and yeast extract/malt extract agar (ISP 2), both at pH 10·0, and used for microscopic observations of the sporophores, spore-chains and spore surface by using light and scanning electron microscopes (JEOL, JSM-5600LV). Cultural characteristics were studied on ISP media (Shirling & Gottlieb, 1966), medium A (Sato et al., 1983), Czapek’s agar (Waksman,
1967), modified Bennet’s medium (Jones, 1949) and nutrient agar (Waksman, 1961). All media were solidified with 2-0% agar and their pH was adjusted to 9.5–10.0; after incubation for 28 days at 28 °C, the colours of both substrate and aerial mycelia and the production of soluble pigments were determined by comparison with chips from ISCC–NBS colour charts (Kelly, 1964).

For chemotaxonomic studies, strain YIM 80379T was grown in medium A broth on a shaking incubator at 200 r.p.m. and 28 °C for 7 days. Mycelia and cells were harvested by centrifugation, washed three times with distilled water and then freeze-dried. Amino acid and sugar analyses of whole-cell hydrolysates were performed as described by Hasegawa et al. (1983) and Stanek & Roberts (1974), respectively. Polar lipids were extracted and detected by previously described methods (Minnikin et al., 1977; Lechevalier & Lechevalier, 1980). Menaquinones were extracted, purified and identified by HPLC as described by Collins (1985).

All physiological tests were done at 28 °C and pH 9.5–10.0 unless otherwise specified. Production of melanoid pigments was tested on ISP media, as described by Shirling & Gottlieb (1966). Carbon source utilization was examined on ISP 9 as a basal medium (Shirling & Gottlieb, 1966), supplemented with a final concentration of 1% of the tested carbon sources (except for sodium acetate, sodium citrate and sodium succinate, which were used at a final concentration of 0.1%). Utilization of different nitrogen sources, catalase production and degradation of tyrosine, hypoxanthine, casein, starch and gelatin were detected in modified Bennett’s agar medium (MBA) after 7, 14 and 21 days, as described by Williams et al. (1983). Hydrogen sulphide production was detected by the method of Küster & Williams (1964). The effect of different temperatures and pH levels on growth and tolerance to salt (NaCl at 5, 10 and 15%, w/v) was determined by using MBA or medium A as a basal medium.

DNA was extracted for 16S rDNA analysis by the method described by Orsini & Romano-Spica (2001). PCR-mediated amplification of 16S rDNA, purification of PCR products and sequencing of purified products were done as described previously (Cui et al., 2001). The resultant sequence was aligned manually against bacterial sequences that were available in public databases. A more detailed comparison was performed with members of the genus Nocardiopsis and evolutionary distance matrices were calculated by the method of Jukes & Cantor (1969). Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

DNA was isolated according to Hopwood et al. (1985) and its G+C content was determined by the thermal denaturation method (Mandel & Marmur, 1968) with a Shimadzu UV–visible spectrophotometer (UV1601). DNA–DNA hybridization was carried out spectrophotometrically, as described by De Ley et al. (1970).

Alkaliphilic strain YIM 80379T showed good growth on most agar media used (Table 1). Aerial mycelium was abundant on most media and its colour varied from white to yellowish-brown. Substrate mycelium was light yellow to yellowish-brown; no soluble pigments were produced on any medium. Mature aerial mycelium fragmented to branched and straight spore-chains with elongated, irregular and smooth spores (Fig. 1).

The isolate’s membership of the genus Nocardiopsis was confirmed by cell chemistry. Whole-cell hydrolysates contained meso-diaminopimelic acid as the only peptidoglycan diamino acid and ribose and glucose as the only sugars, but no diagnostic sugars such as arabinose, xylose, madurose (Lechevalier et al., 1971) or rhamnose (Labeda et al., 1984). This leads to cell wall type III and sugar pattern C (Lechevalier & Lechevalier, 1970). The polar lipid pattern revealed the presence of phosphatidylcholine

### Table 1. Cultural characteristics of strain YIM 80379T

<table>
<thead>
<tr>
<th>Medium*</th>
<th>Growth</th>
<th>Aerial mycelium</th>
<th>Substrate mycelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone/yeast extract (ISP 1)</td>
<td>Poor</td>
<td>–</td>
<td>Light yellow†</td>
</tr>
<tr>
<td>Yeast extract/malt extract (ISP 2)</td>
<td>Moderate</td>
<td>White</td>
<td>Yellowish-brown</td>
</tr>
<tr>
<td>Oatmeal agar (ISP 3)</td>
<td>Abundant</td>
<td>Yellowish-white</td>
<td>Yellowish-brown</td>
</tr>
<tr>
<td>Inorganic salts/starch agar (ISP 4)</td>
<td>Good</td>
<td>White</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Glycerol/asparagine agar (ISP 5)</td>
<td>Good</td>
<td>White</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Tyrosine agar (ISP 7)</td>
<td>Abundant</td>
<td>Yellowish-white</td>
<td>Yellow</td>
</tr>
<tr>
<td>Bennet agar</td>
<td>Moderate</td>
<td>White</td>
<td>Greyish-yellow</td>
</tr>
<tr>
<td>Czapek agar</td>
<td>Abundant</td>
<td>Yellowish-white</td>
<td>Soft yellow</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>Good</td>
<td>Yellowish-white</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Sato A</td>
<td>Abundant</td>
<td>Yellowish-white</td>
<td>Deep yellow</td>
</tr>
</tbody>
</table>

*All media were adjusted to pH 9.5–10.0. ISP, International Streptomyces Project (Shirling & Gottlieb, 1966).
†Colours were taken from ISCC–NBS colour charts (Kelly, 1964).
unknown phospholipids with high amounts of PG, lack of hydroxy-PE and the detection of polyspora with glucose, galactose, lactose, rhamnose, xylitol, sorbitol, good carbon sources, but weak utilization was observed. Maltose, cellobiose, raffinose and sucrose were utilized as no melanoid pigments were produced. Arabinose, xylose, mannose, mannitol and sodium acetate were not utilized. Growth on potassium nitrate, asparagine, phenylalanine and serine as nitrogen sources was recorded, but histidine, methionine, valine, threonine, cysteine and glycine were utilized weakly, whereas growth on arginine and hydroxyproline was not observed. Strain YIM 80379\(^T\) could degrade tyrosine, hypoxanthine, casein, gelatin, starch and tributrin; it also produced catalase, but not H\(_2\)S. Temperature range for growth was 10–45°C; it showed optimum growth at 28–30°C. It grew only on alkaline media. No growth was observed below pH 7-0 and the optimum pH for growth was 9-5–10-0. The maximum pH for growth was 12-0. Good growth was shown at NaCl concentrations up to 10-0 %.

The almost-complete 16S rDNA sequence of strain YIM 80379\(^T\), which consisted of 1490 bp, was determined. Preliminary comparison of the sequence against those in GenBank indicated that members of the genus Nocardiopsis were the closest phylogenetic neighbours. Binary similarity values of this strain and other species of the genus Nocardiopsis ranged between 95-4 % (Nocardiopsis halo- phila DSM 44494\(^T\)) and 98-5 % (Nocardiopsis prasina DSM 43845\(^T\)). Pairwise similarity values > 97 % were also found for Nocardiopsis listeri DSM 40297\(^T\) (98-4 %), Nocardiopsis metallicus DSM 44598\(^T\) (98-2 %), Nocardiopsis exhalans DSM 44407\(^T\) (98-1 %), Nocardiopsis alba DSM 43377\(^T\) (97-8 %), Nocardiopsis lucentensis DSM 44048\(^T\) (97-7 %), Nocardiopsis dassonvillei subsp. dassonvillei DSM 43111\(^T\) (97-7 %), Nocardiopsis umidischolae DSM 43662\(^T\) (97-1 %) and Nocardiopsis synnemataformans DSM 44143\(^T\) (97-2 %). These 16S rDNA sequence similarity values are approximately the same or less than the similarity values between closely related Nocardiopsis species, such as N. dassonvillei and N. synnemataformans (99-3 %), N. metallicus and N. exhalans (99-4 %), N. alba and N. prasina (99-0 %), N. halotolerans and N. dassonvillei (98-4 %) and N. listeri and N. prasina (98-8 %). A phylogenetic tree of Nocardiopsis species is shown in Fig. 2. The closest phylogenetic neighbours of strain YIM 80379\(^T\) are N. listeri DSM 40297\(^T\), N. prasina DSM 43845\(^T\), N. metallicus DSM 44598\(^T\) and N. exhalans DSM 44407\(^T\). These data indicate that strain YIM 80379\(^T\) probably belongs to a novel species. However, sequence similarity values of > 97 % was reported to be of limited usefulness in species differentiation, and DNA pairing studies need to be performed to confirm the species affiliation (Stackebrandt & Goebel, 1994).

DNA of strain YIM 80379\(^T\) was hybridized against that of N. prasina DSM 43845\(^T\), N. listeri DSM 40297\(^T\), N. metallicus DSM 44598\(^T\) and N. exhalans DSM 44407\(^T\), which were the closest phylogenetic neighbours in the same subclade. DNA–DNA relatedness between strain YIM 80379\(^T\) and the latter four strains was 42, 58, 18 and 35 %, respectively. These values are below the value of 70 % that was recommended by Wayne et al. (1987) for strains of the same species. The DNA G+C content of strain YIM 80379\(^T\) was

Strain YIM 80379\(^T\) synthesized a complex pattern of menaquinones with 9, 10 and 11 isoprenoid units in the side chain and a variable degree of saturation. Major menaquinones were MK-10(H\(_6\)), MK-10(H\(_8\)), MK-11(H\(_2\)), MK-9(H\(_6\)), MK-9(H\(_10\)) and MK-10(H\(_4\)). Minor menaquinones were MK-11(H\(_4\)), MK-9(H\(_2\)), MK-9(H\(_4\)) and MK-10(H\(_2\)). Trace amounts of some other menaquinones were also found. This quinone system, with the predominant menaquinones MK-10(H\(_6\)), MK-10(H\(_8\)) and other MK-10 menaquinones, is characteristic of species of the genus Nocardiopsis. Also, such a complex quinone system, or one even more complicated, was reported in earlier studies (Evtushenko et al., 2000; Al-Zarban et al., 2002; Kämpfer et al., 2002). All these characteristics are typical of the genus Nocardiopsis (Grund & Kroppenstedt, 1990; Kroppenstedt, 1992).

No melanoid pigments were produced. Arabinose, xylose, maltose, cellobiose, raffinose and sucrose were utilized as good carbon sources, but weak utilization was observed with glucose, galactose, lactose, rhamnose, xylitol, sorbitol, inositol, dulcitol, sodium citrate and sodium succinate. Ribose, fructose, mannose, mannitol and sodium acetate were not utilized. Growth on potassium nitrate, asparagine, phenylalanine and serine as nitrogen sources was recorded, but histidine, methionine, valine, threonine, cysteine and glycine were utilized weakly, whereas growth on arginine and hydroxyproline was not observed. Strain YIM 80379\(^T\) could degrade tyrosine, hypoxanthine, casein, gelatin, starch and tributrin; it also produced catalase, but not H\(_2\)S. Temperature range for growth was 10–45°C; it showed optimum growth at 28–30°C. It grew only on alkaline media. No growth was observed below pH 7-0 and the optimum pH for growth was 9-5–10-0. The maximum pH for growth was 12-0. Good growth was shown at NaCl concentrations up to 10-0 %.

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![Fig. 1. Scanning electron micrograph of strain YIM 80379\(^T\) after growth on medium A for 14 days at 28°C. Bar, 1 μm.](image-url)
Nocardiopsis alkaliphila (alka.li'phi.la. N.L. n. alkali from Arabic al-qaliy the ashes of saltwort; Gr. adj. philos friendly, loving; N.L. fem. adj. alkaliphila loving alkaline environments).

Table 2. Characteristics that differentiate strain YIM 80379T from the phylogenetically most closely related Nocardiopsis species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Aerial mycelium</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Galactose</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>D-Sucrose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Serine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Degradation of hypoxanthine</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 °C</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45 °C</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>pH 12-0</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Major menaquinones*</td>
<td>10/6, 10/8</td>
<td>10/0, 10/2</td>
<td>10/4, 10/6</td>
<td>10/6, 10/8</td>
<td>ND</td>
</tr>
</tbody>
</table>

*10/6, MK-10 (H6) and so on.
Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is yellow to yellowish-white. Substrate mycelium is yellow to yellowish-brown. Diffusible pigments are not produced. Mature aerial mycelium fragments to branched and straight spore-chains with elongated, irregular and smooth spores. Whole-cell hydrolysates contain meso-diaminopimelic acid and the sugars glucose and ribose. Polar lipid pattern is composed of PG, PG, PME, PE, PIM, DPG, an unknown glycolipid and about four unknown phospholipids with high Rf values. Major menaquinones are MK-10(Ho), MK-10(H4), MK-11(H2), MK-9(H4), MK-9(H10) and MK-10(H4); minor menaquinones MK-11(H4), MK-9(H2), MK-9(H4) and MK-10(H2) are also detected. Melanin is not produced. Arabinose, xylose, maltose, cellobiose, raffinose and sucrose are utilized as good carbon sources, but weak utilization of glucose, galactose, lactose, rhamnose, xylitol, sorbitol, inositol, dulcitol, sodium citrate and sodium succinate is observed. Ribose, fructose, mannose, mannitol and sodium acetate are not utilized. Growth on potassium nitrate, asparagine, phenylalanine, methionine, valine, threonine, cysteine and glycine are utilized only weakly, whereas growth on arginine and serine as nitrogen sources is recorded, but histidine, glutamate and proline are utilized only weakly. Growth on potassium nitrate, asparagine, phenylalanine, methionine, valine, threonine, cysteine and glycine are utilized only weakly, whereas growth on arginine and serine as nitrogen sources is recorded, but histidine, glutamate and proline are utilized only weakly.

The type strain is YIM 80379T (=CCTCC AA001031T = DSM 44657T). Isolated from desert soil in Egypt.

Acknowledgements

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References


