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Characterization of a New Alkaliphilic *Nocardiopsis* Strain from the Desert of Egypt

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An alkaliphilic actinomycete strain, designated WS65, was isolated from a desert soil sample collected from Beni-Suef Governorate in Egypt, and was then subjected to polyphasic taxonomy. The strain produced substrate and aerial mycelia on different media with optimum pH for growth 9.5- 10 and no growth was recorded at pH 7. Strain WS65^T contained *meso*-diaminopimelic acid, no diagnostic sugars, type PIII phospholipids, and MK-10 (H_8) and MK-10 (H_6) as the predominant menaquinones. The morphological and chemotaxonomic characteristics of the isolate were in agreement with those described for members of the genus *Nocardiopsis*. This was confirmed by 16S rRNA sequence comparison and phylogenetic analysis. The strain was distinguished from the closely related *Nocardiopsis* species by a number of phenotypic characters, but the high DNA-DNA pairing similarities didn't support the proposal of strain WS65 (=DSM 44686^T =CCTCC AA001033^T) as a novel species in the genus *Nocardiopsis*. The 16S rRNA sequence of the strain was deposited in GenBank under the accession number AY331687.

Key words: Characterization, *Nocardiopsis*, alkaliphilic, desert, polyphasic taxonomy.

It was reported that members of the genus *Nocardiopsis* characteristically form a well developed and branched substrate mycelium which may fragment into coccoid and bacillary elements [1]. They have a cell wall chemotype III, which means that the strains contain *meso*-diaminopimelic acid, alanine and glutamic acid in their peptidoglycan. In whole cell hydrolysates glucose and galactose are detected, but no diagnostic sugars have been found in any of the species (type C) [2]. The *Nocardiopsis* strains show a characteristic phospholipid type III profile [3] with phosphatidylcholine (PC) as the characteristic polar lipid and a complex menaquinone profiles containing predominant amounts of MK-10(H_0) to MK-10(H_8) and small amounts of the MK-9 and /or MK-11 series [4].

Most *Nocardiopsis* strains are characterized by their alkaliphilic behavior as they prefer mild alkaline conditions and some can even grow at pH 13 [1]. Alkaliphilic species include *Nocardiopsis alkaliphila*, *N. ganjiahuensis, N. litoralis, N. metallicus*, *N. prasina* and *N. valliformis*[5-10]. It was reported also that isolates belong to the genus *Nocardiopsis* can be recovered from the desert soil samples [5, 11, 12].

In the present study, an alkaliphilic actinomycete isolated from a desert soil sample collected from Beni-Suef Governorate, Egypt was identified by polyphasic approach and found to be a member of the genus *Nocardiopsis*.

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MATERIALS AND METHODS

Isolation of the strain

Strain WS65 was isolated from a desert soil sample collected from Beni-Suef Governorate, Egypt by using medium A recommended by Sato *et al*. [13]. After incubation at 28ºC for 14 days, strain WS65 was picked and subcultured until purification. A preliminary test was carried out to confirm its requirement for alkalinity. It was unable to grow below pH7.0. The strain was maintained in 20% glycerol and kept at -20ºC.

Cultural characteristics and microscopic observations

The cultural characteristics were studied on ISP media [14], medium A [13], Czapek's agar [15], Modified Bennet's agar [16], and nutrient agar [15]. The pH of all media used was adjusted to 9.5-10.0, and after incubation for 28 days at 28ºC, the colors of both substrate and aerial mycelia and the production of soluble pigments were determined by comparison with chips from the ISCC-NBS color charts [17]. The isolate cultivated on medium A and yeast extract-malt extract agar (ISP 2) [14], both at pH 10.0, was used for microscopic observations of the sporophores, spore chains and spore surface using light and scanning electron microscopes (JEOL, JSM-5600LV).

Chemotaxonomic studies

For chemotaxonomic studies, the alkaliphilic strain WS65 was grown on medium A broth on a shaking incubator at 200 rpm and 28ºC for 7 days. The mycelia and cells were harvested by centrifugation and washed three times with distilled water and then freeze-dried. The amino acid and sugar analysis of the whole-cell hydrolysates were performed as described by Hasegawa *et al*. [18] and Staneck & Roberts [19], respectively. Polar lipids were extracted and detected by the previously described method [20]. Menaquinones were extracted, purified and identified by HPLC as described by Collins [21].

Physiological and biochemical characteristics

All the physiological and biochemical tests were done at 28ºC and pH 10 with the same media and conditions as described in details elsewhere [5].

Phylogenetic analysis

Genomic DNA was extracted for 16S rRNA analysis by the method described by Orsini & Romano-Spica [22]. PCR-mediated amplification of the 16S rRNA, purification of PCR products and sequence of purified products were done as described previously [23]. The resultant sequence was manually aligned against antibacterial sequences available from 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 [24]. Phylogenetic trees were inferred by using the neighbour-joining [25] and maximum-likelihood [26] methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings [27].

Determination of the G+C content and DNA-DNA similarities

DNA was isolated according to Hopwood *et al*. [28] and the G+C content was determined by the thermal denaturation method [29]. DNA-DNA hybridization was carried out spectrophotometrically as described by De Ley *et al*. [30].

RESULTS AND DISCUSSION

The morphological and cultural characteristics of the alkaliphilic strain WS65 were consistent with those described for *Nocardiopsis* species. It has wide growth capabilities and showed abundant growth on the all used media (Table 1). Its aerial mycelium color varied from yellowish white/grayish yellow pink to grayish red brown. The substrate mycelium color varied from yellow/yellowish brown to grayish red brown. No soluble pigments were produced except in Sato agar medium with light grayish brown color. The spore chains of the mature aerial mycelium were long and branched with elongated smooth spores with variable lengths (Fig. 1). Morphological structures such as synnemata were not observed.

Chemotaxonomically, the whole-cell hydrolysates contained *meso*-diaminopimelic acid, and galactose as the only sugar, but no characteristic sugars (cell wall type III and sugar pattern C). The polar lipid pattern consisted of phosphatidyl choline (PC), phosphatidyl methylethanolamine (PME), phosphatidyl ethanolamine (PE), diphosphatidyl glycerol (DPG) and unknown glucosamine-containing phospholipids (GluNu's). The phospholipid pattern

is type PIII according to Lechevalier *et al*. [3] with PC and PME as the diagnostic phospholipids. The menaquinone pattern was consisted of MK-10(H_8), MK-10(H_6) and MK-9(H_4). This quinone system, with the predominant menaquinones MK-10(H_6), MK-10(H_8) is characteristic for species of the genus *Nocardiopsis*. All of these characteristics are typical of the genus *Nocardiopsis* [31, 32, 1].

The physiological and biochemical characteristics also support the assignment of WS65 to genus *Nocardiopsis*. The melanoid pigments were not produced on any of the media used. It showed a wide range of carbon utilization. It could utilize glucose, arabinose, ribose, xylose, galactose, mannitol, rhamnose, sucrose, maltose, raffinose, cellibiose, inositol, sodium citrate and sodium succinate. Weak utilization was observed with lactose, fructose, xylitol, dulcitol, sorbitol and sodium acetate. Only mannose could not be utilized as a carbon source. Asparagine, histidine and phenylalanine could be utilized as good nitrogen sources. Arginine, valine, glycine, cysteine and potassium nitrate could be utilized as weak nitrogen sources while, hydroxyproline, methionine, threonine, serine and sodium nitrate could not be utilized. It could also degrade tyrosine,

Table 1. Cultural characteristics of strain WS65.

Medium[a]	Growth	Aerial mycelium	Substrate mycelium
Tryptone-yeast extract (ISP1)[b]	Abundant	Brownish pink	Deep yellow[c]
Yeast extract-malt extract (ISP2)	Abundant	Light grayish red brown	Moderate yellow brown
Oatmeal agar (ISP3)	Abundant	Grayish red brown	Grayish red brown
Inorganic salts-starch agar (ISP4)	Abundant	Yellowish white	Pale olive
Glycerol-asparagine agar (ISP5)	Abundant	Yellowish white	Deep yellow
Tyrosine agar (ISP7)	Abundant	Yellowish white	Simple yellow brown
Bennet agar	Abundant	Light grayish red brown	Deep olive brown
Czapek agar	Abundant	Grayish yellow pink	Grayish brown
Nutrient agar	Abundant	Light grayish red brown	Moderate yellow brown
Sato A	Abundant	Light grayish red brown	Grayish brown

[a] All media adjusted to pH 9.5- 10.0;
[b] ISP, International Streptomyces Project (Shirling & Gottlieb, 1966).
[c] Colors were taken from ISCC-NBS COLOR CHARTS (Kelly, 1964).

Table 2. Characteristics differentiating between WS65 and the phylogenetically most closely related *Nocardiopsis* species in its clade.

Character	WS65	*N. synnemataformans*	*N. dassonvillei*
Synnemata	%	+	%
Utilization of:			
L-Arabinose	+	%	+
D-Xylose	+	+	+
D-Mannose	%	+	+
D-Sucrose	+	%	+
D-Maltose	+	+	%
Acetate	±	%	%
Citrate	+	%	+
L-Serine	%	%	±
L-Phenylalanine	+	+	%
Growth at:			
10°C	+	%	%
45°C	+	%	%
pH 12	+	ND	ND

hypoxanthine, casein and adenine well but weak degradation was recorded with tributrin. It grew only on the alkaline media. No growth was observed below or at pH 7 and the optimum pH value for growth was 9.5- 10. The maximum pH value for growth was 12. The temperature range for growth was from 10°C to 45°C. It showed optimum temperature for growth at 28-30°C. It showed also good growth at different concentrations of NaCl up to 10%.

Phylogenetic analysis of the 16S rRNA sequence confirmed the membership of strain WS65 to genus *Nocardiopsis*. Also, the G+C content was 65.41 mol%, which lies within the range for the genus [1]. The sequence similarity values of this strain and other species of the genus

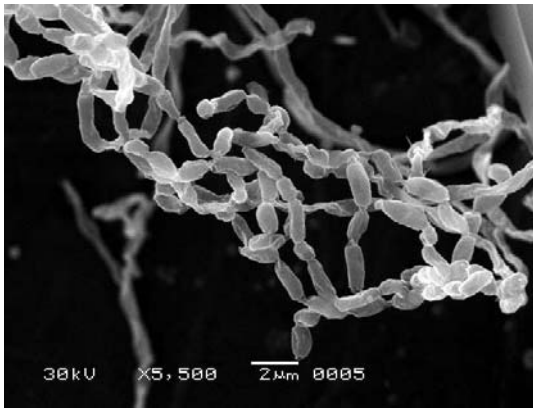

Fig. 1. Scanning electron micrograph of WS65 on ISP2 medium for 14 days at 28°C showing the spore variable lengths and smooth surfaces.

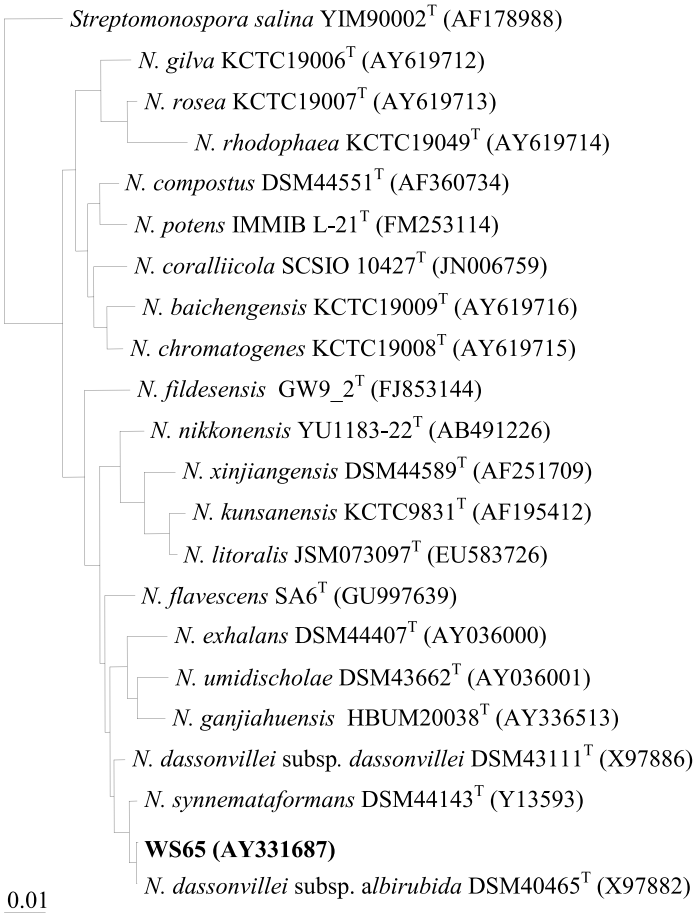

Fig. 2. Phylogenetic tree based on 16S rRNA sequence analysis reconstructed from evolutionary distances using the neighbor-joining method, showing the phylogenetic position of strain WS65 and the closely related *Nocardiopsis* species. The sequence of *Streptomonospora salina* YIM90002^T (AF178988) was used as outgroup. Scale bar, inferred nucleotide substitution per 100 nucleotides and the bootstrap values are not shown.

Nocardiopsis ranged from 96.1% with *N. halophila* to 99.7% with *N. dassonvillei subsp albirubida* and *N. synnemataformans*. The phylogenetic tree of strain WS65 and its relationship to the other closely related *Nocardiopsis* species is shown in Fig. 2.

The phylogenetic position of this strain is within a clade contains the two subspecies of *N. dassonvillei* and *N. synnemataformans*. However, WS65 can be differentiated from those closely related species in this clade by a combination of phenotypic characters (Table 2).

The high values of 16S rRNA sequence similarities were recorded before between the closely related *Nocardiopsis* species, such as *N. dassonvillei* and *N. synnemataformans* (99.4%). This high similarity value leaded Yassin *et al*. [33] to highlight the limited usefulness of 16S rRNA sequence data and the importance of DNA:DNA relatedness to discriminate between closely related *Nocardiopsis* species.

Therefore, the DNA of strain WS65 was hybridized against that of *N. dassonvillei* subsp. *dassonvillei* DSM43111^T, *N. synnemataformans* DSM44143^T and *N. halotolerans* DSM44410^T, the closest phylogenetic neighbours. They share a DNA:DNA pairing values of 65.2%, 35.4 and 18%, respectively. These values, although below the 70% cutoff point recommended for the delineation of genomic species (Wayne *et al*., 1987), but it didn't give enough support in our opinion to describe the *Nocardiopsis* WS65 strain as a new species in the genus *Nocardiopsis*.

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