

## TAXONOMY AND ANTIMICROBIAL ACTIVITIES OF A NEW *STREPTOMYCES* SP. TN17 ISOLATED IN THE SOIL FROM AN OASIS IN TUNIS

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**Abstract** - An actinomycete strain referred to as TN17 was screened for its antimicrobial activities. The taxonomic status of this strain was established. The organism was found to have morphological and chemotaxonomic characteristics typical of Streptomycetes. Based on the 16S rRNA nucleotide sequences, *Streptomyces* sp. TN17 was found to have a relationship with *Streptomyces lilaceus*, *Streptomyces gobitricini* and *Streptomyces lavendofoliae*. Combined analysis of the 16 S rRNA gene sequence (FN687757), phylogenetic analysis, fatty acids profile and physiological tests indicated that there are genotypic and phenotypic differences between TN17 and neighboring *Streptomyces* species' neighbors. Therefore, TN17 is a novel species: *Streptomyces* sp. TN17 (=DSM 42020<sup>T</sup>=CTM50229<sup>T</sup>). A cultured extract of this strain inhibits the growth of several Gram positive and Gram negative bacteria and fungi.

**Key words:** Actinomycete, polyphasic taxonomy, 16S rRNA gene, *Streptomyces* sp. TN17 (=CTM50229<sup>T</sup>=DSM 42020<sup>T</sup>), antimicrobial activities

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### INTRODUCTION

Actinomycetes are gram-positive bacteria that are free-living, saprophytic, widely distributed in soil, water; they also colonize plants, exhibit, marked chemical and morphological diversity and form a distinct evolutionary line of organisms (Goodfellow and O'Donnell, 1989).

At least 90% of actinomycetes isolated from soil have been reported to be *Streptomyces* spp (Anderson and Wellington, 2001). The genus *Streptomyces* was proposed by Waksman and Henrici (1943) and classified in the family *Streptomycetaceae* on the basis of morphology and subsequent characterized cell

wall chemotype. Streptomycete systematics, notably the delineation of species, is becoming increasingly objective due to the application of the polyphasic taxonomic approach. However, the classification of the genus *Streptomyces* in the current edition of Bergey's Manual of Systematic Bacteriology (Willimas et al., 1989) is based not on a combination of genotypic and phenotypic properties, but on the extensive numerical taxonomic survey of Williams et al. (1989). Given the presence of a phylogenetic branching pattern, a combination of properties such as wall chemotype, peptidoglycan type, whole-cell sugars, fatty acid and phospholipid profiles and menaquinones facilitates the delineation of actinomycete genera from neighboring taxa (Kroppenstedt et al., 1990). *Streptomyces*

species can be distinguished from other actinomycetes by their cell wall type which is characterized as Type I *sensu* (Lechevalier and Lechevalier, 1970). The presence of L-L diaminopimelic acid and glycine and the absence of characteristic sugars are typical of this cell wall type (Uchida and Seino, 1997). The analysis of 16S rRNA has proved to be a very important tool in *Streptomyces* systematics, as well as helpful in assigning the newly isolated strain to the genus *Streptomyces*. There is increasing interest in the isolation of a novel *Streptomyces* species as they are very potent producers of active secondary metabolites (Mellouli et al., 2003). *Streptomyces* have been the most fruitful source of microorganisms for all types of bioactive metabolites that have important applications in human medicine and in agriculture fields (Watve et al., 2001).

During our routine screening program for the isolation of novel actinomycete bacteria producing bioactive compounds, an interesting bacterium, referred to as TN17, was isolated from Tunisian oasis soil samples and selected for its capacity to produce antimicrobial molecules. In the present work, we describe the identification of a *Streptomyces* strain, designated as TN17, isolated from a Tunisian oasis soil sample by conventional and molecular methods, as well as the antimicrobial activities of the culture extract of this strain.

## MATERIALS AND METHODS

### *Strain isolation and conservation*

Strain TN17 was isolated by the dilution agar plaiting method from oasis soil from southern Tunisia. The strain was maintained by cultivation on an ISP 2 agar medium that contained (per liter) 4 g glucose, 4 g yeast extract, 5 g malt extract and a vitamin/amino acid mixture (1 mg vitamin B1; 1 mg vitamin B2; 1 mg vitamin B6; 1 mg biotin; 1 mg nicotinic acid; 1 mg phenylalanine; 0.3 g alanine) at pH 7.2, incubated at 28°C for two weeks. The strain was maintained on a yeast extract-malt extract-dextrose (YMD) agar medium at 4°C (Williams and Cross, 1971).

### *Phenotypic characterization*

The cultural characteristics and the colors of the mature sporulating aerial mycelium and the substrate mycelium of the isolated TN17 were monitored in 7, 14 and 21 day-old cultures grown in different agar media as follows: the Four (ISP 2-5) International Streptomyces Project (ISP) media recommended by Shirling and Gottlieb (1966); the Bennett agar medium; the Nutrient agar medium and the Sabouraud agar medium. Melanin production was tested in the peptone-yeast extract-iron (ISP6 medium) agar and trypsin (ISP7 medium) agar.

### *Chemotaxonomic studies*

Sufficient biomass for chemotaxonomic studies was obtained after incubation at 28°C for 3 days by growing in shake-flasks in ISP 2 broth. The isomeric form of diaminopimelic acid (DAP), glycine and sugars in the whole cell hydrolyzates were analyzed by TLC (Staneck and Roberts, 1974). Phospholipids were examined by two-dimensional TLC and identified using several spray reagents and by comigration with standards (Collins and Jones, 1980; Minnikin et al., 1979). Fatty acids were extracted, methylated and analyzed by gas chromatography (GC) using the standard Microbial Identification System (MIDI) (Sasser 1990).

### *Physiological tests*

The ability of the strains to utilize 15 compounds as sole carbon sources and 16 compounds as sole nitrogen sources for energy and growth was examined on specimens grown on ISP medium 9 for 3 days at 28°C. Each source was added at a final concentration of 1% (w/v) and 0.1% (w/v), respectively. The utilization of sole carbon and sole nitrogen sources was investigated according to Shirling and Gottlieb (1966). Sodium salts (acetate, alginate, benzoate, butyrate, citrate, desoxycholate, hydrogen carbonate, nitrate, oxalate, perchlorate, propionate, pyruvate, succinate, sulfite, tartrate, tetraborate and thiosulfate) were added at a final concentration of 0.1% (w/v) (Gordon et al., 1974).

Esculin and arbutin (1.0%, w/v) degradation was determined by the methods of Williams et al. (1989) and examined after 3 days incubation of the TN17 strain in ISP2 solid medium. The degradation of casein (1.0%, w/v) was detected in the ISP2 agar after either 3, 7 and 14 incubation days and clearing under and around the colonies' growth areas was scored as positive. Gelatin (0.4%, w/v) and starch (1.0%, w/v) degradation was read after 3 days in the ISP2 agar by flooding the plates with trichloroacetic acid (3.0%, v/v) and iodine solutions respectively, and scoring the zones of clearing as positive (Williams et al., 1989).

The degradation of tyrosine, hypoxanthine, xanthine, adenine and guanine (1.0%, w/v) was investigated according to Gordon et al. (1974) and the hydrolysis of Tween 80 was measured using the method of Sierra (1957).

The effects of salt on growth were determined in TSB media supplemented with graded doses of sodium chloride (1, 4, 5, 7 and 10% w/v). Maximum sodium chloride concentration in the medium allowing any growth was recorded (Williams et al., 1989).

The growth at various temperatures was tested using TSA plates incubated at 4, 10, 15, 25, 30, 37, 40 and 45°C. The effects of pH on growth were tested in pH-adjusted TSB media (pH 4.0~10.0 in 0.5 unit increments).

Tolerance to lysozyme (0.005%), phenol (0.05, 0.1%, 0.2%, 0.5% and 1.0%), and sodium azide (0.001 and 0.01%) was tested using GYEA media (Athalye et al., 1985).

Resistance to antibiotics was examined with erythromycin, streptomycin, penicillin at (10 mg/l), rifampicin, gentamicin, vancomycin at (5 mg/l) and chloramphenicol, oxytetracycline, kanamycin at (25 mg/l) incorporated into the glucose yeast extract agar (Lechevalier and Lechevalier a, b, 1970) as a basal medium. Readings were taken at 1, 3, 7 and 14 days of growth. Organisms were scored as resist-

ant (+) when growth on the test plates was greater or equal to that on positive control plates lacking inhibitors.

#### *Genotypic characterization*

The genomic DNA of strain TN17 was isolated as described by Hopwood et al. (1985). PCR amplification of the 16S rRNA gene of strain TN17 was performed in an automated thermocycler (Perkin Elmer) using two primers 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATC-CAGCCGCA-3' as described by Edwards et al. (1989) and according to the amplification profile described by Elleuch et al. (2010). The PCR reaction mix was analyzed by agarose gel electrophoresis and DNA of the expected size was purified and then cloned into a pCR-Blunt vector. The nucleotide sequence of the 16S rRNA gene of strain TN17 was determined on both strands by an automated 3100 Genetic Analyzer (Applied Biosystems) using specific primers. For phylogenetic analysis, reference strains were chosen from the BLAST (Altschul et al., 1997) results. The nucleotide sequence of the whole 16S rRNA gene (1521 pb) of TN17 strain has been assigned in GenBank (EMBL) under accession number FN687757. Multiple sequence alignment was carried out using CLUSTAL W (Thompson et al., 1997) at the European Bioinformatics Institute website (<http://www.ebi.ac.uk/clustalw/>). Phylogenetic analyses were performed using programs from the PHYLIP package (Felsenstein, 1985) and a phylogenetic tree was constructed by the neighbor joining (NJ) algorithm (Saitou and Nei, 1987) using Kimura 10-parameter distance. The robustness of the inferred tree was evaluated by bootstrap (100 replications).

#### *Antimicrobial activities determination*

Indicator microorganisms were grown overnight in LB medium at 30°C for *Micrococcus luteus* LB14110 and at 37°C for *Staphylococcus aureus* ATCC 6538 (Gram-positive bacteria), *Pseudomonas aeruginosa* ATCC 49189, *Salmonella enterica* ATCC43972 and *Escherichia coli* ATCC 8739 (Gram-negative bacte-

ria) and then diluted 1:100 in LB medium and incubated for 5 h under constant agitation of 200 rpm at the appropriate temperature. *Fusarium sp.* was grown in a potato dextrose agar (PDA) for 7 days at 30°C. Spores were collected in sterile distilled water and then adjusted to a spore density of approximately  $10^4$  spores/ml. *Candida tropicalis* R2 CIP203 was grown in YP10 medium (10 g/l yeast extract, 10 g/l peptone, 100 g/l glucose, 15 ml of 2 g/l adenine solution) at 30°C for 24 h in an orbital incubator with shaking at 200 rpm.

Spores of Strain TN17 at  $10^7$ /ml were used to inoculate a 500 ml Erlenmeyer flask with four indents containing 100 ml of TSB (Tryptic Soy Broth) medium at 30 g/l. After incubation at 28°C for 24 h, this pre-culture was used to inoculate at 1/10 (v/v) 1000 ml Erlenmeyer flask with four indents, containing 200 ml of TSB medium. After incubation at 28°C for 72 h in an orbital incubator with shaking at 200 rpm, the culture broth was centrifuged to remove the biomass. The cell-free supernatant was extracted with ethyl acetate (2×) and the obtained organic extract concentrated *in vacuo* to dryness. The resulting dry extract was recuperated in 2 ml of ethyl acetate and assayed against indicator microorganisms. Antimicrobial activities were determined by the agar diffusion test: a paper disk (8mm Ø) was impregnated with 50 µl of the corresponding sample and then laid on the surface of an agar plate containing 3ml of top agar seeded by 40 µl of a 5-h-old culture of the corresponding microorganism. For antifungal activity against the *Fusarium sp.*, 100 µl of spore suspension was added to 3 ml of the top agar. After 2 h at 4°C, plates were incubated overnight at the appropriate growth temperature of the corresponding indicator microorganism. Plates were examined for evidence of antimicrobial activities represented by a zone of inhibition of growth of the studied indicator cell around the paper disk. The experiment was carried out simultaneously three times under the same conditions. In each case, all obtained diameters of inhibition zones were similar and the reported inhibition zones (mm) are the average from three experiments.

## RESULTS AND DISCUSSION

### *Morphological and physiological characteristics*

Strain TN17 is a Gram-positive bacterium. Morphological observation of the 7–15 day-old culture of this strain grown on yeast extract-malt extract agar (ISP2) (Shirling and Gottlieb, 1966) revealed that both aerial and vegetative hyphae were abundant. The isolate developed well on several media, including ISP2, ISP3, ISP4, ISP5, Bennett agar and nutrient agar media. The detailed cultural characteristics of strain TN17 are given in Table 1. The aerial mycelium was abundant, well-developed and varied from white to gray on all tested media. The substrate hyphae varied from yellowish-white to yellowish-brown. Diffusible pigments were not produced on any test media and melanin was not produced. The physiological features are indicated in Table 2 and in the species description.

The strain *Streptomyces sp.* TN17 has been deposited in the DSMZ and CTM under the numbers DSM 42020<sup>T</sup> and CTM50229<sup>T</sup>, respectively.

### *Chemotaxonomic analysis*

The cell wall peptidoglycan of strain TN17 contained only LL-diaminopimelic acid and glycine, indicating that this strain has a chemotype cell-wall type I (Lechevalier and Lechevalier a, b, 1970). Whole-cell hydrolyzates contained mainly glucose and small quantities of xylose, galactose and arabinose. The diagnostic phospholipid was phosphatidylethanolamine (PE) (phospholipids type II sensu (Lechevalier and Lechevalier, 1970b)). The fatty acid profile included mainly saturated iso- and anteiso-branched-chains and straight-chain fatty acids “fatty acid type 2c sensu” (Kroppenstedt, 1985). The major cellular fatty acids were iso  $C_{15:0}$  (23.58%), anteiso  $C_{15:0}$  (29.88%) and iso  $C_{16:0}$  (28.52%), and smaller amounts of  $C_{12:0}$  3-OH (1.61%),  $C_{15:0}$  (3.12%),  $C_{16:0}$  (4.49%),  $C_{16:1}$  w 9 (3.57%) and iso  $C_{17:0}$  (3.89%) were also present.

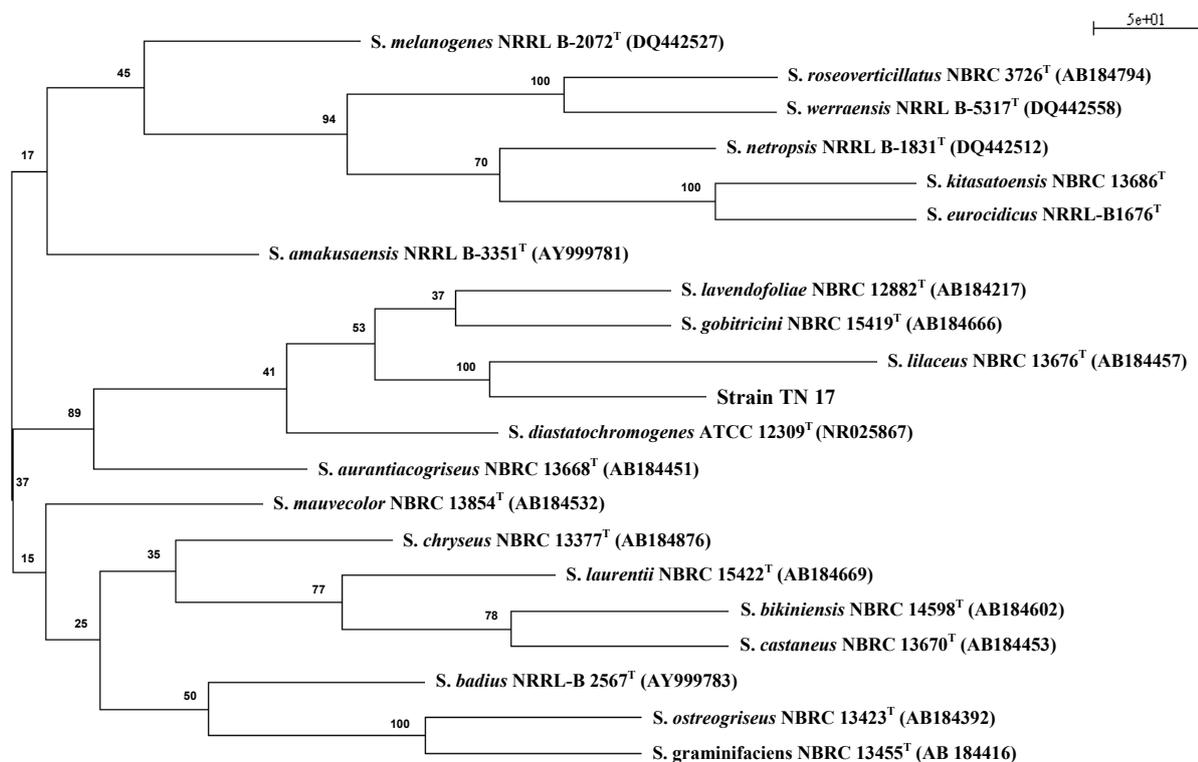


Fig. 1. Phylogenetic trees of the *Streptomyces* sp. TN17 strain.

The chemical and morphological properties of strain TN17 are clearly consistent with its assignment to the genus *Streptomyces* (Williams et al., 1989).

#### Phylogenetic analysis

The nucleotide sequence of the 16S rRNA gene of strain TN17 was determined on both strands. The nucleotide sequence of the whole 16S rRNA gene (1521 pb) of TN17 strain has been assigned GenBank (EMBL) under accession number FN687757. This sequence was subjected to similarity searches against public databases to infer possible phylogenetic relationships of strain TN17. The phylogenetic tree (Fig. 1) from representative strains of the related species indicated that strain TN17 should be placed in the genus *Streptomyces*. In the comparison of 16S rRNA gene sequences, TN17 was mostly related with *S. lilaceus* NBRC 13676<sup>T</sup> (99.79%), *S. gobitricini* NBRC 15419<sup>T</sup> (99.66%), and *S. lavendofoliae* NBRC 12882<sup>T</sup> (99.52%).

#### Physiological characteristics

A comparative study between strain TN17 and closely related species of the genus *Streptomyces* revealed that it differed from *S. lilaceus* NBRC 13676<sup>T</sup>, *S. gobitricini* NBRC 15419<sup>T</sup> and *S. lavendofoliae* NBRC 12882<sup>T</sup> in morphological, cultural, and physiological characteristics as summarized in Table 2. In addition, the aerial mycelium of strain TN17 varied from white to gray and soluble pigments were not produced. In contrast, the substrate mycelium of *S. lilaceus* NBRC 13676<sup>T</sup> is grayish reddish brown to strong brown, and soluble yellow or red pigments are produced. The aerial mycelium of *S. lavendofoliae* NBRC 12882<sup>T</sup> and *S. gobitricini* NBRC 15419<sup>T</sup> are grayish yellow and soluble pigments were not produced.

For comparative studies, the physiological characteristics of related type strains were tested together with that of strain TN17. On the basal

**Table 1.** Culture characteristics of strain TN17 in different media.

Medium	Growth	Sporulation	Aerial mycelium	Substrate mycelium
Yeast -malt extract agar (ISP <sup>T</sup> medium 2)	Good	Good	Gray	Soft yellowish brown
Oatmeal agar (ISP <sup>T</sup> medium 3)	Good	Good	White	Pale yellow
Inorganic salt-starch agar (ISP <sup>T</sup> medium 4)	Good	Good	White	Soft yellowish white
Glycerol-asparagine agar (ISP <sup>T</sup> medium 5)	Good	Good	Gray	Moderate brown
Bennett agar	Good	Good	Gray	Pale yellow
Nutrient agar	Good	Moderate	Gray	Soft yellow
Sabouraud agar	Moderate	Moderate	Gray	Pale yellow

**Table 2.** Physiological properties separating strain TN17 from related *Streptomyces* species. Strains: 1, TN17; 2, *Streptomyces lavendofoliae* NBRC 12882<sup>T</sup>; 3, *Streptomyces lilaceus* NBRC 13676<sup>T</sup> and 4, *Streptomyces gobitricini* NBRC 15419<sup>T</sup>.

Characteristics	1	2	3	4
Colony color on ISP2	gray	greyish yellow	greyish yellow	gray
Production of diffusible pigment	-	-	-	-
Melanin production on ISP6	-	-	+	-
Melanin production on ISP7	-	-	+	-
Melanoid pigment on tryptone-yeast extract broth	-	-	+	-
Nitrate reduction	+	+	+	+
<i>Growth on sole carbon sources (1%, w/v)</i>				
L-Arabinose	+	+	-	+
D-Fructose	-	+	-	-
D-Galactose	+	-	+	+
Glucose	+	+	+	+
Glycerol	+	-	+	+
Meso-Inositol	-	+	+	-
D-Lactose	-	-	+	+
Maltose	+	+	+	+
D-Mannose	-	-	-	+
D-Raffinose	-	-	-	-
L-Rhamnose	-	-	-	-
D-Ribose	+	-	-	+
Sucrose	+	-	-	-
D-Trehalose	+	-	-	+
D-Xylose	-	+	-	+
<i>Growth on sole energy sources (0.1%, w/v)</i>				
Sodium acetate	+	+	+	-
Sodium alginate	-	+	-	-
Sodium benzoate	-	+	-	-
Sodium butyrate	-	-	+	+
Sodium citrate	+	+	+	+
Sodium desoxycholate	-	+	+	-
Sodium hydrogen carbonate	-	+	-	-
Sodium nitrate	-	-	+	+
Sodium oxalate	-	+	-	+
Sodium perchlorate	+	-	+	+
Sodium propionate	-	-	+	+
Sodium pyruvate	-	+	+	-
Sodium succinate	+	+	-	-
Sodium sulfite	-	+	-	-

**Table 2.** Continued

Characteristics	1	2	3	4
Colony color on ISP2	gray	greyish yellow	greyish yellow	gray
Sodium tetraborate	-	+	+	-
Sodium thiosulfate	+	-	+	+
<i>Growth on sole nitrogen sources (0.1%, w/v)</i>				
L-Asparagine	-	+	-	+
L-Aspartic acid	-	-	-	-
L-Alanine	+	+	+	+
L-Arginine	+	-	+	-
L-Cysteine	+	-	-	+
L-Glutamic acid	-	-	-	-
L-Histidine	+	+	+	-
L-Isoleucine	+	-	+	-
L-Leucine	+	-	+	-
L-Methionine	+	+	+	+
L-Phenylalanine	+	-	+	-
L-Proline	+	-	+	+
L-Serine	+	+	+	+
L-Threonine	-	-	-	-
L-Tryptophane	-	+	-	-
L-Tyrosine	-	+	+	-
<i>Degradation of</i>				
Adenine	-	-	-	-
Arbutin	-	+	-	-
Casein	+	+	+	+
Esculin	-	+	+	-
Gelatin	+	+	+	-
Guanine	-	-	-	+
Hypoxanthine	+	+	+	+
Starch	+	+	+	+
Tween 80	+	+	+	+
Xanthine	-	+	-	+
<i>Growth temperature (°C) range</i>	25-40	30-37	25-40	30-40
<i>Growth pH range</i>	5-9	5-9	5-8	5-8
<i>Growth in the presence of:</i>				
Lysozyme (0.005%)	+	+	+	+
Phenol (0.05%)	+	+	+	+
(0.1%)	+	+	+	+
(0.2%)	+	+	+	-
(0.5%)	-	-	+	-
(1.0%)	-	-	-	-
Sodium azide (0.001%)	-	+	-	+
(0.01%)	-	-	-	-
<i>Growth sodium chloride range (%)</i>	1-7	1-7	1-5	1-5
<i>Resistance to antibiotics (µg/ml)</i>				
Erythromycin (10)	-	-	-	-
Streptomycin (10)	+	+	-	-
Penicillin (10)	-	-	-	-
Gentamicin (5)	+	+	-	+
Rifampicin (5)	-	+	+	-
Vancomycin (5)	+	-	-	-
Chloramphenicol(25)	-	-	-	-
Oxytetracycline (25)	+	-	+	-
Kanamycin (25)	-	+	+	-

**Table 3.** Cellular fatty acid contents (%). 1 - strain TN17; 2 - *Streptomyces lavendofoliae* NBRC 12882<sup>T</sup>; 3 - *Streptomyces lilaceus* NBRC 13676<sup>T</sup>; 4 - *Streptomyces gobitricini* NBRC 15419<sup>T</sup>

Fatty acids	1	2	3	4
C <sub>10:0</sub> 2-OH	-	-	-	-
C <sub>11:0</sub>	-	0.753	-	-
C <sub>12:0</sub>	0.094	0.1244	0.358	-
C <sub>12:0</sub> 2-OH	0.026	0.466	-	-
C <sub>12:0</sub> 3-OH	1.611	-	1.344	2.221
C <sub>13:0</sub>	-	1.447	0.145	0.285
C <sub>14:0</sub>	0.453	0.26	0.615	0.634
C <sub>14:0</sub> 2-OH	0.152	0.235	-	-
C <sub>14:0</sub> 3-OH	-	-	-	-
C <sub>15:0</sub>	3.120	0.126	0.9272	1.344
Iso C <sub>15:0</sub>	23.583	43.728	23.193	24.382
anteiso C <sub>15:0</sub>	29.889	44.306	29.859	31.467
C <sub>16:0</sub>	4.490	1.356	5.404	3.896
Iso C <sub>16:0</sub>	28.529	3.994	29.595	24.454
C <sub>16:0</sub> 2-OH	-	-	-	-
C <sub>16:1</sub> <i>w</i> 9	3.578	-	3.488	4.029
C <sub>17:0</sub>	0.146	-	-	4.228
Iso C <sub>17:0</sub>	3.891	2.533	4.344	2.802
anteiso C <sub>17:0</sub>	0.3508	-	0.3715	0.2535
C <sub>18:0</sub>	-	-	0.352	-
C <sub>18:1</sub> <i>w</i> 9 (cis)	0.0109	-	-	-
C <sub>18:1</sub> <i>w</i> 9 (trans)	-	-	-	-
C <sub>18:2</sub> <i>w</i> 9,12	0.0701	-	-	-
C <sub>19:0</sub>	-	-	-	-
Iso C <sub>19:0</sub>	-	-	-	-
C <sub>20:0</sub>	-	-	-	-

**Table 4.** Antimicrobial activities of the crude extract of the supernatant culture of *Streptomyces* sp. TN17

Test organism	Diameter of inhibition zones (mm)
<i>Micrococcus luteus</i> LB14110	21
<i>Staphylococcus aureus</i> ATCC 6538	18
<i>Salmonella enterica</i> ATCC43972	14
<i>Escherichia coli</i> ATCC 8739	-
<i>Pseudomonas aeruginosa</i> ATCC 49189	-
<i>Fusarium</i> sp.	18
<i>C. tropicalis</i> R2 CIP203	11

medium (Pridham and Gottlieb, 1984), TN17 utilized L-arabinose, D-galactose, glucose, glycerol, maltose, D-ribose, sucrose and D-trehalose but not D-fructose, meso-inositol, D-lactose, D-mannose, D-raffinose, D-rhamnose and D-xylose as

sole carbon sources. This utilization was different from the patterns of all strains used for comparison (Table 2). The four strains of *Streptomyces* have the sameas physiological characters such as the ability to utilize sole carbon and nitrogen sourc-

es: glucose, maltose, D-mannose, D-raffinose and L-rhamnose (sole carbon sources) and L-aspartic acid, L-alanine, L-glutamic acid, L-methionine, L-serine and L-threonine (sole nitrogen sources).

Growth of strain TN17 was observed at a wide range of temperature (25–40°C), although the optimal temperature range was between 25 and 30°C. The initial pH range for which growth of strain TN17 was observed was between pH 5-9; however, the optimal pH value for growth was determined to be 7.5. Strain TN17 was also capable of growth in the presence of 7% NaCl and 0.2% of phenol. In addition, the strain TN17 reduced nitrate to nitrite. Casein, gelatin, starch, hypoxanthine and Tween 80 were degraded by *Streptomyces* sp. TN17 but not adenine, arbutin, esculin, guanine and xanthine.

The detailed fatty acid profile of strain TN17 given in Table 3 was clearly different from that of *Streptomyces lavendofoliae* NBRC 12882<sup>T</sup>, *Streptomyces lilaceus* NBRC 13676<sup>T</sup> and *Streptomyces gobitricini* NBRC 15419<sup>T</sup>. For *Streptomyces lilaceus* NBRC 13676<sup>T</sup>, the major cellular fatty acids were iso C<sub>15:0</sub> (23.19%), anteiso C<sub>15:0</sub> (29.85%) and iso C<sub>16:0</sub> (29.59%) and smaller amounts of C<sub>16:0</sub> (5.4%), C<sub>16:1</sub> w 9 (3.48%) and Iso C<sub>17:0</sub> (4.34%). For *Streptomyces gobitricini* NBRC 15419<sup>T</sup>, the major cellular fatty acids were iso C<sub>15:0</sub> (24.38%), anteiso C<sub>15:0</sub> (31.46%) and iso C<sub>16:0</sub> (24.45%) and smaller amounts of C<sub>12:0</sub> 3-OH (2.22%), C<sub>16:0</sub> (3.89%), C<sub>16:1</sub> w 9 (4.02%), C<sub>17:0</sub> (4.22%) and iso C<sub>17:0</sub> (2.8%). For *Streptomyces lavendofoliae* NBRC 12882<sup>T</sup> the major fatty acids were iso C<sub>15:0</sub> (43.72%) and anteiso C<sub>15:0</sub> (44.3%) and smaller amounts of iso C<sub>16:0</sub> (3.99%) and iso C<sub>17:0</sub> (2.53%).

Based on the genotypic and phenotypic evidence, it is suggested that strain TN17 is a novel species of the genus *Streptomyces*, for which the name is *Streptomyces* sp. TN17 (=DSM 42020<sup>T</sup>=CTM50229<sup>T</sup>).

#### *Biological activities*

As shown in Table 4, the ethyl acetate extract of the supernatant culture of the *Streptomyces* sp TN17 exhibited an inhibitory effect against *M. luteus* LB14110,

*S. aureus* ATCC 6538 (Gram positive bacteria), *S. enterica* ATCC43972 (Gram negative bacterium), *Fusarium* sp. (filamentous fungus) and *C. tropicalis* R2 CIP203 (Yeast).

#### CONCLUSION

An aerobic bacterium TN17 was isolated from the oasis soil of Tunisia. The bacterium has morphological characteristics and chemotaxonomic properties consistent with its assignment to the genus *Streptomyces*. This strain was compared phenotypically and phylogenetically with the nearest species in the genus *Streptomyces*: this comparison suggests that they are different from *Streptomyces* species.

*Streptomyces* sp. TN17 (=DSM 42020<sup>T</sup>=CTM50229<sup>T</sup>) showed antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi.

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