International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (1), pp. 001-006, January, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Actinopolyspora egyptensis sp. nov., a new halophilic actinomycete

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Accepted 22 December, 2018

A halophilic actinomycete, designated HT371^T, was isolated from a soil sample collected from the shore of the salty Lake Qaroun, Egypt, and was the subject of a polyphasic study. Analysis of 16S rRNA indicated that the isolate belonged to the genus *Actinopolyspora* and constituted a separate clade in the *Actinopolyspora* 16S rRNA gene tree with similarity values of 96.5 and 96.2% with *Actinopolyspora halophila* DSM43834^T and *Actinopolyspora mortivallis* DSM44261^T, respectively. Isolate HT371^T had chemotaxonomic and morphological properties consistent with its classification in the genus *Actinopolyspora* and could grow on agar plates at NaCl concentrations of up to 25% (w/v). The isolate was readily differentiated from the type strains of genus *Actinopolyspora* using a range of phenotypic characters. On the basis of polyphasic evidence, the strain HT371^T represents a novel species for which the name *Actinopolyspora egyptensis* sp. nov. is proposed. The type strain is HT371^T (=CGMCC 4.2041^T).

Key words: *Actinopolyspora egyptensis* sp. nov., halophilic isolate, polyphasic taxonomy.

INTRODUCTION

The genus *Actinopolyspora* was created by Gochnauer et al. (1975) to harbour *Actinopolyspora halophila* on the basis of morphological and chemotaxonomic properties. The genus currently comprises three validly described species, namely *A. halophila* (Gochnauer et al., 1975), *Actinopolyspora iraqiensis* (Ruan et al., 1994) and *Actinopolyspora mortivallis* (Yoshida et al., 1991). All the *Actinopolyspora* species are halophilic, which can grow best between 10 and 20% NaCl, and therefore Johnson et al. (1986) suggested that salterns and adjacent environments may be appropriate habitats to investigate for the presence of novel *Actinopolyspora* strains. During a study on halophilic actinomycetes in the area of Lake Qaroun, Egypt which is an enclosed saline inland lake, a novel strain was isolated that had the characteristics reported for members of the genus *Actinopolyspora*. The present investigation was designed to determine its taxonomic status, which showed that it merited recognition as a new species of the genus *Actinopolyspora*, for which the name *Actinopolyspora egyptensis* sp. nov. is proposed.

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MATERIALS AND METHODS

Isolation and maintenance of the organism

Strain HT371^T was isolated from a soil sample collected from the shore of the salty Lake Qaroun in Egypt, which was serially diluted and plated onto a complex medium (CM) agar plate (7.5 g casamino acids, 10 g yeast extract, 20 g $MgSO_4.7H_2O$, 3 g sodium citrate, 2 g KCl, 150 g NaCl, 18g agar, 1 l distilled water, pH 7.4) after incubation at 28^oC for 14 days. The organism was then maintained on CM slants at room temperature and as a glycerol suspension of hyphae and spores (20%, v/v) at -20°C.

Chemotaxonomical markers

The biomass required for the chemotaxonomic and molecular systematic studies, derived from a 7-day-old CM broth shake culture incubated at 28°C and 150 rpm, was harvested by

centrifugation, washed twice with distilled water and freeze-dried in case of chemotaxonomic studies till required. Isolate HT371^T was the subject of chemotaxonomic analyses designed to confirm its generic assignment. Standard procedures were used to extract and analyze the isomeric forms of diaminopimelic acid (Hasegawa et al., 1983), whole- organism sugars (Staneck and Roberts, 1974), muramic acid type (Uchida et al., 1999), isoprenoid quinones (Minnikin et al., 1984; Collins, 1994), polar lipids (Minnikin et al., 1984) and fatty acids (Sutcliffe, 2000).

Phylogenetic analysis of the 16S rRNA gene sequence

Extraction of chromosomal DNA, polymerase chain reaction (PCR) amplification and direct sequencing of 16S rRNA from isolate HT371^T were carried out as described previously (Chun and Goodfellow, 1995). The resultant sequence was aligned manually with corresponding sequences of the type strains of the genera classified in the family *Pseudonocardiaceae*, as retrieved from the DDBJ/EMBL/GenBank databases, using known 16S rRNA secondary structure information. Phylogenetic trees were constructed by using the least-squares (Fitch and Margoliash, 1967), maximum-parsimony (Kluge and Farris, 1969) and neighbor joining (Saitou and Nei, 1987) tree-making algorithms. Evolutionary distance matrices for the least-squares and neighbor-joining methods were generated after Jukes and Cantor (1969). The resultant unrooted tree topology was evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. The Clustal X program version 1.8 (Thompson et al., 1997) and the software package MEGA version 3.1 (Kumar et al., 2004) were used for the multiple alignment and the phylogenetic analyses.

Morphological and cultural characteristics

Morphological characteristics of the undisturbed arrangement of hyphae, especially aerial hyphae and spore chains, were examined on CM agar after 7 to 30 days at 28°C by light microscope using the coverslip technique of Kawato and Shinobu (1959). Spore arrangement and spore surface ornamentation were observed by examining gold coated dehydrated preparation using a Cambridge Stereoscan 240 scanning electron microscope and the procedure described by O'Donnell et al. (1993). The cultural characteristics of the isolate, notably aerial spore mass colour, substrate mycelial pigmentation and the colour of any diffusible pigment were observed using the methods and media of the ISP (Shirling and Gottlieb, 1966) in addition to modified Bennett's agar (Jones, 1949), CM and nutrient agar after incubation for one month at 28°C. All media were supplemented with 15% NaCl. Gram (Hucker's modification, Society of American Bacteriologists, 1957) and Ziehl-Neelsen (Gordon, 1967) preparations were examined following growth on CM agar for 7 to 30 days at 28°C by light microscope.

Phenotypic characteristics

Strain HT371^T and the three type strains of genus *Actinopolyspora*, *A. halophila* DSM 43834^T, *A. iraqiensis* DSM 44640^T and *A. mortivallis* DSM 44261^T, were examined for a range of phenotypic properties. The NaCl requirement for growth was determined on CM agar supplemented with different concentrations of NaCl. Utilization of different sole carbon compounds for energy and growth, degradation and hydrolysis of various organic compounds by the strains under test were carried out using established methods (Shirling and Gottlieb, 1966; Gordon and Mihm, 1962; Williams et al., 1983). The ability to grow at different temperatures and Ph values was tested on CM agar. All tests were done in triplicate; the media were supplemented by 15% NaCl except in case of *A. iraqiensis* where 10% NaCl was used and the results were recorded following incubation for 7 to 30 days at 30°C.

RESULTS AND DISCUSSION

The membership of the isolate at the genus level was confirmed by its chemical characteristics. The organism was characterized by the presence of *meso*-diaminopimelic acid, arabinose and galactose in whole organism hydrolysates (wall chemotype IV *sensu* Lechevalier and Lechevalier, 1970), N-acetyl muramic acid residues, tetrahydrogenated menaquinone with nine isoprene units as the major isoprenologue and phosphatidyl choline (PC), diphosphatidyl glycerol (DPG), phosphatidyl glycerol (PG), phosphatidyl inositol (PI), phosphatidyl inositol mannosides (PIM) and phosphatidyl methyl ethanolamine (PME) as major polar lipids (phospholipid type PIII *sensu* Lechevalier et al., 1977). The major fatty acid components were *anteiso*-C17:0 (39.7%), *i*-C15:0 (22.2%), *iso*-C17:0 (10%), *iso*-C16:0 (9.5%) and *anteiso*-C15:0 (6.9%) (fatty acid type 2c *sensu* Kroppenstedt, 1985). This chemical profile is consistent with the assignment of strain HT371^T to the genus *Actinopolyspora* (Embley et al., 1988; Gochnauer et al., 1989; Embley, 1992).

A comparison of the sequence of isolate HT371^T with those of representatives of genera classified in the family *Pseudonocardiaceae* showed that it fell within the evolutionary radiation occupied by the genus *Actinopolyspora*. Strain HT371^T formed an independent separate phyletic line within the clade containing *Actinopolyspora* species, which was supported by a high bootstrap value (Figure 1). Sequence similarity calculations after neighbour-joining analysis indicated that the sequence similarity values of this strain and the two other species with available sequences of the genus *Actinopolyspora* are 96.5 and 96.2% with *A. halophila* DSM43834^T and *A. mortivallis* DSM44261^T, respectively. It is obvious from phylogenetic analyses based on almost complete 16S rRNA sequences that isolate HT371^T belongs to the genus *Actinopolyspora* and represents a distinct phyletic line that can be equated with genomic species (Stackebrandt and Goebel, 1994).

The organism is aerobic, Gram-positive and acid fast. Morphological observations of the 7 to 30 days-old culture of strain HT371^T grown on CM agar revealed that it produced well developed and branched substrate mycelium which fragments at maturity. The sporophores were branched and the aerial mycelium formed long chains of spores. The spores were elongated to rod-shaped with variable lengths (1 to 2 m) and had smooth surfaces. The cultural characteristics of strain HT371^T are given in Table 1. Growth was abundant to moderate on most tested media and no diffusible pigments were produced on any medium.

The results of the phenotypic studies are given in Table 2

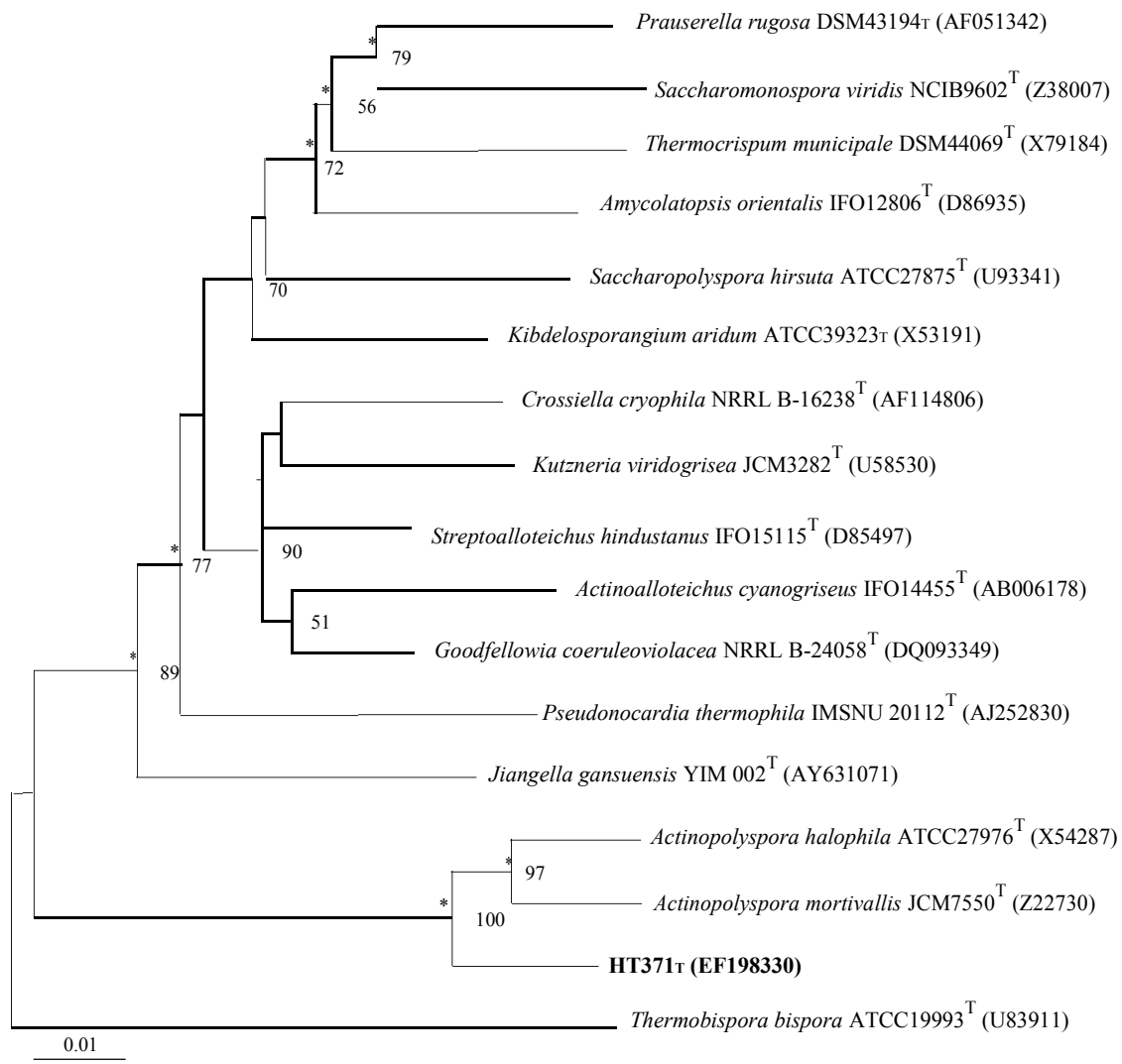

Figure 1. Neighbour-joining tree (Saitou and Nei, 1987) based on 16S rRNA gene sequences showing relationships between isolate HT371^T and representatives of the family *Pseudonocardiaceae*. Asterisks indicate branches of the tree that were also recovered using the least-squares (Fitch and Margoliash, 1967) and maximum-parsimony (Kluge and Farris, 1969) tree-making algorithms. Numbers at the nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50% are given. Bar, 0.01 substitutions per nucleotide position.

and in the species description. Strain HT371^T grows on CM with NaCl concentrations between 5 and 25%; no growth was detected in absence of NaCl. The isolate grows optimally at 15 to 20% NaCl; and at 35°C and pH 7 in media supplemented with 15% NaCl. It is evident from Table 2 that strain HT371^T can be differentiated readily from the type strains of *Actinopolyspora* using a combination of phenotypic features. It was distinguished from all other strains by its inability to utilize mannitol and citrate as sole carbon sources for growth and energy and by its ability to produce acids from cellobiose, erythritol and maltose.

It is evident from the genotypic and phenotypic data presented here that isolate HT371^T belongs to genus *Actinopolyspora*, however, it is phylogenetically distant and exhibits distinctive phenotypic characteristics that differentiate it from other species in the genus. Therefore, based on a polyphasic evidence, isolate HT371^T merited the classification as a new species in the genus *Actinopolyspora* for which the name *A. egyptensis* sp. nov. is proposed.

Description of *Actinopolyspora egyptensis* sp. nov.

A. egyptensis (e.gyp.t.ensis M.L. adj. *egyptensis*, from Egypt, the source of the strain). Aerobic, Gram-positive, acid fast, non-motile actinomycete which forms a

Table 1. Cultural characteristics of strain HT371^T.

Medium[a]	Growth	Aerial mycelium	Substrate mycelium
Tryptone-yeast extract (ISP1)[b]	Weak	White [c]	Greyish yellow
Yeast extract-malt extract (ISP2)	Moderate	Pale yellow pink	Light brown
Oatmeal agar (ISP3)	Good	Yellowish white	Pale yellow
Inorganic salts-starch agar (ISP4)	Abundant	Pale yellow pink	Light yellowish brown
Glycerol-asparagine agar (ISP5)	Good	Yellowish white	Greyish yellow
Bennett's agar	Moderate	Pale yellow pink	Light yellowish brown
Nutrient agar	Weak	---	Moderate yellow
CM	Abundant	Pale yellow pink	Light yellowish brown

[a]All media were supplemented with 15% NaCl. [b] ISP, International *Streptomyces* Project (Shirling and Gottlieb, 1966).
[c]Colors were taken from ISCC-NBS COLOR CHARTS (Kelly, 1958).

Table 2. Phenotypic characteristics that differentiate strain HT371^T from the other *Actinopolyspora* species.

Character	**HT371^T**	***A. halophila* DSM 43834^T**	***A. iraqiensis* DSM 44640^T**	***A. mortivallis* DSM 44261^T**
Spore chains	**Long**	**Long**	**Short**	**Long**
Utilization of sole carbon sources:				
L-Arabinose	–*	–	+	+
D-Cellobiose	+	–	+	+
Dulcitol	+	–	+	+
D-Mannitol	–	+	+	+
D-Mannose	–	±	–	±
D-Melezitose	+	–	+	+
D-Raffinose	–	D	+	+
Salicin	–	±	+	–
D-Sorbitol	–	–	+	–
Xylitol	+	±	+	–
D-Xylose	–	D	+	±
Sodium citrate	–	+	+	+
Acid production from:				
D-Cellobiose	+	–	–	–
i-Erythritol	+	–	–	–
D-Glucose	+	+	–	+
Maltose	+	–	–	–
D-Mannitol	–	–	+	–
Sucrose	**–**	**–**	**–**	**+**
Degradation of:				
Casein	–	+	–	+
Hypoxanthine	–	+	–	–
Starch	+	+	–	+
Growth at:				
5% (w/v) NaCl	+	–	+	+
20% (w/v) NaCl	+	+	–	+
25% (w/v) NaCl		+	–	+
10°C	+	+	–	–
45°C	+	–	–	+
pH9	–	–	–	+

* +, positive; –, negative; ±, weak growth; D, doubtful.

branched substrate mycelium that fragments at maturity. Aerial hyphae differentiate into long chains of elongated or rod-shaped spores with variable lengths (1 to 2 m) and smooth surfaces. Pale yellow to light brown substrate mycelia that carry yellowish white to pale yellowish pink aerial hyphae are formed on different synthetic media. Diffusible pigments are not produced. Growth between 5 and 25% NaCl, 10 and 45˚C, optimally around 15 to 20% NaCl and at 35˚C. Starch is hydrolysed but aesculin, allantoin and arbutin are not. None of casein, hypoxanthine, uric acid or xanthine are degraded. D-cellobiose, dulcitol, *i*-erythritol, D-fructose, D-galactose, D-glucose, maltose, D-melezitose, sucrose, D-trehalose and xylitol are used as sole carbon sources for energy and growth, but not L-arabinose, *meso*- inositol, D-mannitol, D-mannose, melibiose, D -raffinose, L-ribose, D-salicin, D-sorbitol or D-xylose (all at 1%, w/v). Sodium acetate and sodium citrate are not used as sole carbon sources (at 0.1%, w/v). Whole-organism hydrolysates contain *meso*-diaminopimelic acid and the sugars arabinose and galactose. Contains PC, DPG, PG, PI, PIM and PME as major polar lipids, MK-9 (H_4) as the characteristic menaquinone and major proportions of the fatty acids *anteiso*-C17:0 (39.7%), *i*-C15:0 (22.2%), *iso*-C17:0 (10%), *iso*-C16:0 (9.5%) and *anteiso*-C15:0 (6.9%). The type and only strain is HT371^T (=CGMCC 4.2041^T), was isolated from a soil sample collected from the shore of the salty Lake Qaroun, Egypt.

ACKNOWLEDGEMENTS

The authors are grateful to Professor R.M. Kroppenstedt (DSMZ) for kindly providing type strains of *Actinopolyspora*. WH is grateful for support from the Center of Excellence for Biodiversity Research, College of Science, King Saudi University, Riyadh, Saudi Arabia which is highly appreciated.

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