Characterization of a novel strain of the genus *Actinopolyspor*a, an extremely halophilic actinomycete isolated from Saudi Arabia

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Abstract: Actinomycetes have attracted a great attention due to their ability for production of various useful secondary metabolites. . In these work, an extremely halophilic filamentous actinomycetes was isolated from hyper saline soil sample collected from Jeddah region in the west of Saudi Arabia. This isolate can grow at high NaCl concentration up to 30% (w/v). Phylogenetic studies using16S rDNA gene sequence analysis was performed in order to specify the isolate. Results showed that morphological, physiological and biochemical characteristics of the isolate were matched to the genus *Actinopolyspora*. Basing on phylogenetic studies and searching the isolate against the EMBL public database, the isolate is proposed as novel strain of the genus *Actinopolyspora*. The name *Actinpolyspora saudiensis* sp. nov. is proposed and the aim of the study was to describe the isolation, morphology, physiology and biochemical characteristics of this novel strain. [Manal J. Kiki, Idriss M. Al Turk]. **Characterization of a novel strain of the genus *Actinopolyspor*a, an extremely halophilic actinomycete isolated from Saudi Arabia.** *Life Sci J* 2013;10(1):603-608]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 97

Keywords: *Actinopolyspora, halophilic actinomycete, saline soil, halophile, 16S rDNA sequencing.*

1. Introduction

The occurrence of actinomycetes in high saline environments and the tolerance of these organisms to high concentration of salt have been described (Tresner et al., 1968; Gottlieb, 1973). The genus *Actinopolyspora* was extremely halophilic actinomycetes and first proposed by Gochnauer and co-workers (Gochnauer et al., 1975). Since 1975, many species were described. These are *A. halophila* (Gochnauer et al., 1975),*A. motivallis* (Yoshida et al., 1991), *A. iraqiensis* (Ruan et al., 1994), *A. xinjiangensis* (Guan et al., 2010), *A. alba* and *A. erythre*a (Tang et al., 2011) and *A. egyptensis* (Hozzein and Goodfellow , 2011).

All the *Actinopolyspora* species are halophilic, which can grow best between 10 and 20 % NaCl, and can be isolated frequently, probably due to the high occurrence of these actinomycetes in the hyper saline soil environment. Originally, the genus *Actinopolyspora* was assigned to the family Nocardiaceae. It requires high NaCl concentration for growth and can grow in saturated NaCl (Zhi et al., 2007).

In this work, an actinomycete isolated from a saline soil in the west of Saudi Arabia which grew best in the presence of high NaCl concentration (up 30 %). We described morphological, physiological, biochemical and 16S rDNA gene sequence of the isolate which support identification of this isolate as new strain of the genus *Actinopolyspora*. The name *A. saudiensis* sp. nov is proposed for this organism.

2. Materials and Methods

2.1. Isolation of the microorganism

Serial dilution was carried out for the bacterial strain and an aliquot was plated on starch nitrate agar. After incubation at 40 ºC for 14 days, the isolate was streaked for pure culture and then maintained on starch nitrate slant agar at 4 ºC.

2.2. Cultural and morphological characteristics

The cultural characteristics of the isolate, notably aerial spore mass color, substrate mycelial pigmentation and the color of any diffusible pigment were determined after 2-3 weeks by methods and media used in the ISP (Shirling and Gottlieb, 1966). All media were supplemented with 15% NaCl.

The morphological characteristics of cell and spore were determined using scanning electron microscope (Philips XL20) after 14 days of growth on yeast extract malt extract agar supplemented with 15% Na Cl. The isolate was fixed with glutaraldehyde, dehydrated by using a graded ethanol and acetone series, critical point – dried, then gold-coated as described (Gabriel, 1982).

2.3. Physiological characteristics

All the tests were carried out at 40 ºC, unless otherwise specified. Media and procedure used for physiological and biochemical features and carbon sources utilization were as described (Shirling and Gottlieb, 1966; Locci, 1989 and Chun et al., 2000).

- **The NaCl tolerance** and requirement for growth was determined using starch nitrate agar supplemented with different concentration of NaCl (10, 15, 20, 25, and 30 %).

- **The ability of the strain to grow at different temperatures** (30, 35, 40, 45, 50, 55 ºC) and pH were determined after 7-21 days using starch-nitrate agar supplemented with 15 % NaCl.

- **The utilization of various carbohydrates as sole carbon sources** was examined using basal medium (ISP-9) containing 9 different carbohydrates at a final concentration of 1% (w/v).

- **Hydrolysis of starch and cellulose** were determined respectively on inorganic salt starch medium and carboxymethyl cellulose agar (CMC).

- **Amylolytic and cellulytic activities** were determined by the formation of clear zone, using Lugol (Cowan, 1974) and Congo red (Ariffian et al., 2006) solutions as indicators, respectively.

- **Degradation of casein and tyrosine** were tested using milk agar (Smibert and Krieg, 1994) and tyrosine agar (Shirling and Gottlieb, 1966) respectively.

- **Lipase production** was determined by the method previously described (Maria et al., 2005).

- **Liquefaction of gelatin** was tested using bouillon gelatin broth as described (Arai, 1975).

- **Milk coagulation and peptonization** was detected using skimmed milk medium.

- **Antimicrobial activity** was tested by using agar plate diffusion method according (Greenwood, 1989) against gram (+) bacteria: MRSA, MSSA, *Bacillus subtilis* and gram (-) bacteria: *E. coli*, *Pseudomonas aeruginosa*. Yeast and mould: *Candida albicans*, *Aspergillus niger* and *A. fumicitus*.

2.4. Analysis of 16S rDNA gene sequences

2.4.1. 16S rDNA extraction, amplification and sequencing

Extraction of chromosomal DNA, polymerase chain reaction (PCR) amplification and direct sequencing of the 16S rDNA gene from actinomycete isolate were carried out as described previously (Patel et al., 2000).

2.4.2 Sequence data analysis

The resultant 16S rDNA gene sequence of the isolate was aligned manually with the sequences of members of the order *Actinomycetales* using known 16S rDNA secondary structure information. Sequence data were analyzed with MicroSeq software (Clustal W). The unknown sequence was initially compared to all of the sequence in the MicroSeq (Applied Biosystems) and EMBL databases. The MicroSeq database is a commercial database that primarily consists of type strain sequences. The EMBL database is a public database that contains a large number of sequences, including 16S rDNA gene sequences. Phylogenetic tree was constructed by using neighbor-joining method (Saitou and Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbor-joining data (Flesenstein, 1985).

3. Results and Discussion

3.1. Cultural characteristics

The cultural characteristics of the Actinomycete isolate on various media are given in (Table1). The isolate grew well on inorganic salt-starch medium, the growth was moderate on yeast extract – malt extract (Fig. 1) and tyrosine agar media, no growth was observed on glycerol-aspargine and oat meal media. Diffused melanin pigments were observed on tyrosine agar medium. (All media fortified with 15 % NaCl).

Figure 1. Cultural characteristics of the actinomycete isolate (U1) grown on yeast extract-malt extract agar (ISP2) for 14 days at 40 ºC.

Table1. Cultural characteristics of Actinomycete isolate (U1).

Media	Growth	Aerial mycelium	Substrate mycelium	Soluble pigments
Yeast extract-malt extract Agar (ISP 2)	moderate	white	yellow	-
Glycerol-aspargin agar (ISP 5)	-	-	-	-
Inorganic salt- starch agar (ISP 4)	good	creamy	creamy	-
Oat meal agar (ISP 3)	-	-	-	-
Tyrosine agar	moderate	yellow	light brown	brown

ISP: International Streptomyces Project (Sharling and Gottlieb, 1966).

3.2. Morphological characteristics

Morphological observations of 7-14 old culture of actinomycete isolate (U1) grown on yeast extract-malt extract agar revealed that it Produced well developed branched substrate mycelium which fragments at maturity. The sporophores were branched and the aerial mycelium formed long spore chain. The spores were elongated to rod shaped with variable length and have smooth surfaces (Fig 2-3).

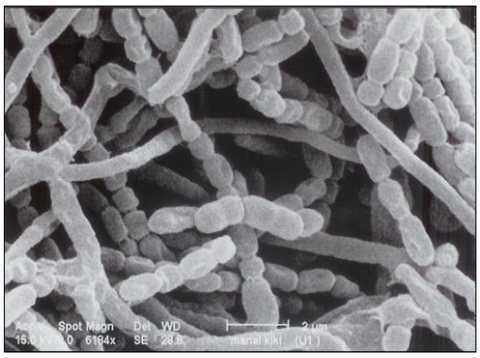

Figure 2. Scanning electron micrograph of aerial mycelium of the isolate (U1) grown on yeast extract malt extract agar supplemented with 15% NaCl for 14 days at 40 ºC. (X 9277).

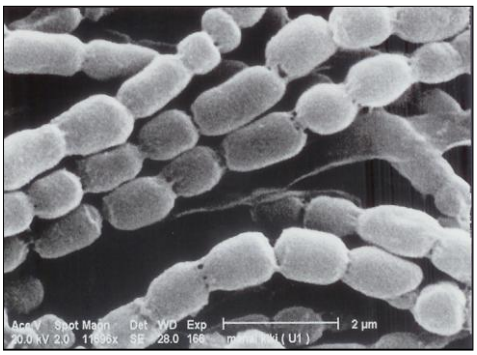

Figure 3. Scanning electron micrograph of a spore chain of the isolate (U1) grown on yeast extract malt extract agar supplemented with 15% NaCl for 14 days at 40 ºC. (X 11696).

3.3. Physiological characterization

The optimum growth temperature of the isolate (U1) was 40 ºC. No growth occurred at 55ºC and the upper limit of growth temperature was 50 ºC, which indicates that it's a moderate thermophilic bacterium. This finding is in agreement with that in Hochstein report (1988) who suggested that most halophiles isolates come from warm climates and many of them can be considered (moderate thermophiles).

Though halophilic archaebacteria are usually grown at 37 ºC. Their optimal temperature for growth is probably higher with values of up to 50 ºC being reported.The *Actinomycete* isolate (U1) grow well at pH 5-6.

The *Actinomycete* isolate was found to be resistant to the inhibitory effects of wide range of NaCl concentrations with the optimal growth ranging between 25-30%. Accordingly the isolate in our study was an extremely halophilic bacterium according to the definition of halophiles (Kushner, 1993).

The strain can produce high actively enzymes, e.g. protease, α-amylase, cellulose, lipase and gelatinase. It was able to decompose starch (Fig. 4). Cellulose, casein, lipids (Fig. 5), coagulation and peptonization of skimmed milk, and the Gelatin was liquefied well by the isolate (U1). The enzymatic activities results of the isolate (U1) are indicated in (Table 2**)**. No antimicrobial activities were detected against Gram (+ve), Gram (-ve) bacteria, yeast and fungi.

3.4. Carbohydrate utilization

The ability of the isolate (U1) to assimilate different carbon sources is presented in (Table 3).The strain assimilated well most of the tested carbohydrate as sole carbon sources for growth suggests a wide range pattern of carbon sources assimilation. The isolate was able to utilize glucose, fructose, cellulose, starch, sucrose, xylose as carbon source, but galactose was poorly utilized and lactose was not utilized**.** The results of phenotypic studies are summarized in (Table 4).

Figure 4. Starch hydrolysis test by actinomycete isolate (U1) grown on Inorganic salt- starch agar (ISP4) for 7 days at 40 ºC.

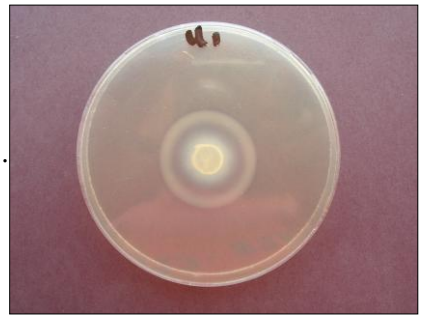

Figure 5. Lipolytic activity by actinomycete isolate (U1) grown on peptone agar for 7 days at 40 ºC.

Table 2. Enzymatic activities of actinomycete isolate (U1)

Reaction	Media	Reaction zone (mm)
Starch hydrolysis	Inorganic salt starch agar	30
Cellulose hydrolysis	Carboxymethyl cellulose	19
Casein hydrolysis	Milk agar	26
Lipolytic activity	Peptone agar media	40
Gelatin liquefactions	Bouillon gelatin broth	+++
Milk coagulation	Skim milk	+++
Milk peptonization	Skim milk	+++

Table 3. Utilization of carbon sources by actinomycete isolate (U1)

Carbon Sources	Utilization
Cellulose	++
Fructose	++
Glucose	++
Galactose	±
Lactose	-
Maltose	+
Starch	++
Sucrose	++
Xylose	++

Table 4. Phenotypic characteristics of actinomycete isolate (U1)

Characteristics	Reaction
Melanin formation	+
NaCl tolerance	30 %
Temperature range for growth	30-50 ºC
Optimum growth temperature	40 ºC
Maximum growth temperature	50 ºC
pH range	5-11
Optimum pH	6
Antimicrobial activity	-
Starch hydrolysis	+
Hydrolysis of carboxymethyl cellulose	+
Casein degradation	+
Milk coagulation	+
Milk peptonization	+
Lipolytic activity	+
Gelatin liquefaction	+

3.5. 16S rDNA sequence analysis

Searching the MicroSeq database did not give a genus level match and therefore the sequences obtained for the isolate was searched against the EMBL public database. Preliminary comparison of the sequence of the isolate against the EMBL was most closely related to members of the genus A*ctinopolyspor*a and was most closely related to *Actinopolyspora mortivallis* (Fig.6).

Sequence similarity calculation after neighbor-joining analysis indicated that the sequence similarity values of this strain is 98.2 % with *Actinopolyspora mortivallis*. This is not considered high enough to be a species level match. It is obvious from phylogenetic analysis based on nearly (500 Pb 16S rDNA sequence) that our isolate belongs to the genus *Actinopolyspora* and represented a distinct phyletic line that can be equated with genomic species (Stackebrandt and Goebel, 1994) considering that high degrees of 16S rDNA gene sequence similarity (97% and higher) have been demonstrated to be of limited value for differentiating species.

3.6. Identification

It is evident from genotypic and phenotypic data previously presented that isolate (U1) belonged to genus *Actinopolyspora* as *Actinopolyspora* sp., however, it is phylogenetically distant and exhibits distinctive phenotypic characteristics that differentiated it from other species in the genus. Therefore, basing on a polyphasic evidence, isolate (U1) merited the classification as new species in the genus *Actinopolyspora* for which the name *A. saudiensis* sp. nov. is proposed.

4. Conclusion

Actinopolyspora saudiensis (Saudi. ensis M.L adj. saudiensis referring to Saudi; the source of the soil sample from which the organism was isolated. Its characteristics were; it is aerobic, Gram positive, non-motile, filamentous *Actinomycete* which forms branch substrate mycelium that fragments at maturity. The aerial mycelium formed chain of spores. These spores are elongated or rod-shaped with variable length. The aerial mycelium is white to creamy yellow and the substrate mycelium is yellow to light brown on different tested synthetic media. Diffused melanoied pigments were observed on tyrosine agar medium. Grows on media supplemented with between 10 - 30% NaCl (w/v).

The organism is extremely halophilic according to the definition (Kuchner, 1993). Optimum growth at 40 ºC and pH 6 with 25-30 % NaCl. Temperature, pH and NaCl tolerance ranges are 20-50 ºC, PH 5-11 and 10-30 % NaCl respectively. No antimicrobial activities were detected. The range of carbon sources utilization of the isolate was wide. Glucose, fructose, cellulose, starch, sucrose, xylose could be utilized as carbon sources, but galactose was poorly utilized and lactose was not utilized**.** Starch hydrolysis, carboxymethyl cellulose degradation, lipolytic activity, gelatin liquefaction, milk coagulation and milk peptonization were positive. Strain was isolated from a saline soil sample collected from Jeddah province at the west of Saudi Arabia.

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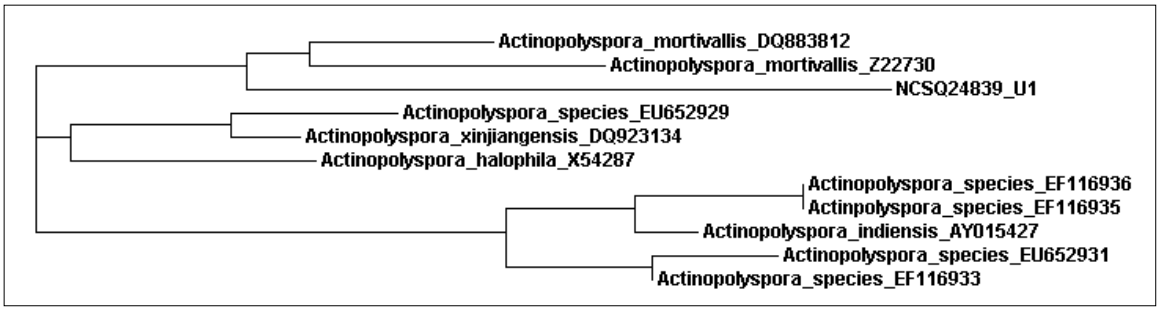

Figure 6. Neighbor-joining tree based on 16S rDNA sequences showing relationships between isolate (U1) and members of the genus *Actinopolyspora*. Only values above (50 %) are given. (EMBL) database.

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