Distribution and Characterization of Actinomycetes in Suez Bay Sediments, Egypt

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ABSTRACT--- ***Sediment samples were collected seasonally (from winter to autumn 2012) from ten sampling sits along the sea shore of Suez Bay for the purpose of studying the distribution of actinomycetes and its relation with the physicochemical parameters. Multiple regression analysis was applied to reflect the relationship between the actinomycetes counts and the environmental factors of sediments during the different seasons. Seventeen actinomycetes isolates were selected to represent all colony morphologies observed on the starch nitrate agar plates. Pure isolates were then subjected to morphological, physiological and biochemical characterization (phenotypic characterization). Phenotypic characters were coded in a binary form of the presence/absence type. Analysis of the results by numerical techniques using the simple matching coefficient (S_SM) and the unweighted pair group method with arithmetic mean (UPGMA) grouped the isolates into two main clusters, cluster A (3 isolates) and cluster B (11 isolates), in addition to single clustered isolates (3 isolates). Seven out of the seventeen isolates (40% of the isolates) exhibited antimicrobial activities against different pathogenic indicators. Genotypic characterizations of the most potent isolates were performed using 16S sequence analysis, where the isolates under study were identified as Streptomyces parvus, Streptomyces griseorubens (cluster A) and Kocuria palustris (cluster B).***

Keywords---- actionomycetes, *Streptomyces parvus, Streptomyces griseorubens, Kocuria palustris,* antibacterial

1. INTRODUCTION

Marine environments present an invaluable source of new natural products that may hold important leads for future drug discovery and development (**Montaser and Luesch, 2011).** These environments are still in their infancy for isolation of new microbes that can produce pharmaceutically valuable metabolites **(Sharma et al., 2013**). Microorganisms including actinomycetes and bacteria are prolific producers of bioactive substances. There are already some detailed reviews on the capabilities and bioactive potential of marine microorganisms (**Bhatnagar and Kim, 2010; Hong et al., 2009**). The highest proportion of bioactive compounds was discovered from actinomycetes **(Hu et al., 2015).** From ancient times actinomycetes are known to produce secondary metabolites that are being used to treat many infections. Many new species of actinomycetes that have not yet been cultured under laboratory conditions are expected to occur in these environments, and they can be a source of drugs with novel chemistry and promising potential (**Sharma et al., 2013).**

Many microbiologists believe that free-living bacteria are cosmopolitan due to their easy dispersal **(Finlay, 2004**). However, chemical and physical factors contribute to selection of species and strains that are best adapted to that particular environment. Some of the unusual structures and properties of compounds isolated from marine sources and the fact that 58 % of the isolated actinomycetes from sediments required sea water for growth (**Jensen, 2005**) implies that one may find microorganisms adapted to the marine environment and producing compounds not found among microorganisms adapted to the terrestrial sources. (Bredholt et al. 2008).

Actinomycetes are filamentous Gram positive bacteria, characterized by a complex life cycle belonging to the phylum Actinobacteria, which represents one of the largest taxonomic units among the 18 major lineages currently recognized within the Domain Bacteria **(Sharma, 2014; Ventura et al., 2007).** Actinobacteria, characteristically high guanine and cytosine (GC≥55%) are the dominant phyla of bacteria found on almost natural substrates **(Usha et al., 2011; Hogan, 2010;)**. They play an important role in decomposition of organic materials and carbon cycle. The taxonomy of the actinomycetes has been subject to unending controversy because of its filamentous, branching growth which resembles with a fungal type of morphology **(Sharma, 2014)**.

Actinomycetes are chemically rich sources of structurally diverse secondary metabolites. **(Kurtböke et al., 2015; Hu et al., 2015; Isik et al., 2014)**. Actinomycetes are economically and biotechnologically most valuable prokaryotes.

Medical or economic significant. Actinobacteria mainly lies in subclass Actinobacteridae, order Actinomycetales. Actinomycetes and their bioactive compound show antibacterial and antimicrobial against various pathogens and multi drug resistant pathogens e.g. Vancomycin-Resistant Enterococci, Methicillin-Resistant *Staphylococcus aureus* , *Shigella dysenteriae*, *Klebsiella sp.* and *Pseudomonas aeruginosa* **(Antunes, 2014; Bhatnagar and Kim, 2010; Servin et al., 2008;)** Actinomycetes are responsible for the production of about a half of the discovered bioactive secondary metabolites, notably antibiotics **(Kurtböke et al., 2015; Antunes, 2014; Usha et al., 2010; Bredholt et al., 2008**), antitumor compounds **(Kurtböke et al., 2015; Usha et al., 2010)**, immune suppressive agents **(Kurtböke et al., 2015)** and enzymes **(Sharma, 2014)**.

The vast majority of these secondary metabolites are mainly derived from members of the genus *Streptomyces* **(Fenical and Jensen, 2006)**. Approximately, 7,600 compounds are produced by *Streptomyces species (***Sharma, 2014; Berdy, 2005)**. The bioactive secondary metabolites from marine-derived *Streptomyces* have thus attracted increasing interest during the last decades **(Blunt et al., 2011)**.

The literature search showed that there were few isolation studies on actinomycetes of the seashore of Egypt until now **(Hamed, 2013; Abou-elela, 1999)**. Therefore, the present preliminary study was aimed to evaluate the distribution and characterization of marine actinomycetes in Suez-Bay sediments, Egypt.

2.MATERIALS AND METHODS

2.1. Sampling sites

Sediment samples were collected seasonally (from winter to autumn 2012) from ten sampling sits along the sea shore of Suez Bay, Egypt (Figure 1). These are: The entrance channel (St.I), El-Kornesh (St.II), Marine high school (St.III), El-zitiat (St.IV), Kabanon (St.V), Electric power station (St.VI), National Institute of Oceanography and Fisheries (St.VII), Attaka (St.VIII), Adabyia (St.IX) and Ayoun mossa (St.X). Samples were immediately transported to the laboratory in an ice box for bacteriological analysis which was always completed within 24 h **(Abou-elela, 1999)**.

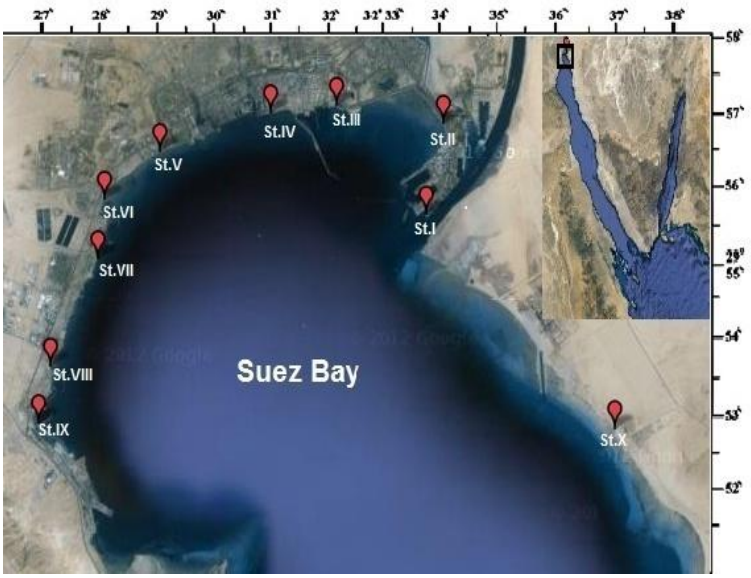

Figure 1: The study area and sampling site

2.2. Actinomycetes isolation and enumeration

Sediments samples without previous dilutions were used, 10 g from each sample were added to 30 ml sterilized sea water, then they were shacked for 20 min, and subjected to thermal treatment (by heating in a water bath at 50 °C for 1h) to reduce the number of unicellular bacteria in favor of actinomycetes . Plates of starch nitrate agar medium **(Ghanem et al., 2000)** were inoculated with 1 ml of the samples. The medium was supplemented with 75 and 25 μg ml^{-1} of filter sterilized cycloheximide and nystatin, respectively, to minimize fungal contamination **(Jensen et al., 1991)**. Enumeration was done on starch nitrate agar plates after 7-14 days incubation at 30-32 °C. Data are expressed as **colony forming unit (CFU g^{-1}).**

2.3. Physicochemical characterization of sediment samples

Physical parameters as temperature, pH and salinity were measured in sediment samples. Also chemical characters as dissolved oxygen **(Clesceri et al., 2012**), dissolved phosphate (**Grasshoff, 1976)**, dissolved nitrate **(Strickland and**

Parsons, 1968), dissolved nitrite **(Grasshoff, 1976)**, dissolved ammonia **(Koroleff, 1969)** and organic matter **(Ellis et al., 1946)** were measured using standard methods.

2.4. Multiple regression analysis:

Multiple regression analysis was applied to reflect the relationship between the actinomycetes counts and the environmental factors of sediments during the different seasons. Multiple regression analysis was performed by MiniTab 16 for all variable and observation. The data were statistically analyzed according to **Steel and Torrie (1960).**

2.5. Morphology, physiology and biochemical characterization

Phenotypic characteristics were determined for the selected isolate according to the standard methods (**Ventosa et al., 1982**). Determination of morphological traits and colours of aerial and substrate mycelium as well as soluble pigments, was done as described by **Shirling and Gottlieb (1966)**. Biochemical tests were performed according to **Ariffin et al. (2006); Cheesbrough (1985), Mostafa (1985), Hankin and Anagnostakis (1975), Weyland et al. (1970), Tresner et al. (1968), and Küster (1959).**

2.6. Screening for antimicrobial activity

2.6.1. Pathogenic indicators:

The bacterial pathogens used as target strains in the present investigation were *Aeromonas hydrophila*, *Vibrio damsela*, *Pseudomonas aeruginosa*ATCC6739, *Escherichia coli* and *Staphylococcus aureus*ATCC6538. These pathogens were kindly provided by the staff members of The National Institute of Oceanography and Fisheries (NIOF), Egypt.

2.6.2. Antibacterial activity

The actinomycetes isolates that were studied were inoculated in flasks (250 mL) containing 50mLof starch nitrate medium. Seeded flasks were incubated at 30°C–32 °C on a rotary shaker at 200 rpm for 7 days. At the end of the incubation, the cultures were harvested, filtered and sterilized by ultrafiltration by using 0.22 µL sterilized filters, and used as antimicrobial agents. The antagonistic activity of actinomycetes' supernatants was detected by using well-cut diffusion technique, in which, five-millimeter diameter wells were punched in marine nutrient agar (Oxoid LTD, England) plates inoculated with bacterial pathogenic strains. Fifty microliters of the tested supernatant was pipetted into each well. The plates were incubated at 30^{o}C for 24h. Each set was prepared in duplicate. After incubation, the radius of the clear inhibition zone around each well was linearly measured in mm **(Cwala et al., 2011; El-Masry et al., 2002).**

2.7. Numerical analysis

Taxonomic characters were coded in a binary form of the presence/absence type **(Sneath and Sokal, 1973**). Data were examined using the SYStat-Pc program **(Wilkinson et al., 1992)**, Similarities among the tested strains were estimated with simple matching coefficient (SsM) **(Sokal and Michenu, 1958)**, The unsorted data were sorted using the unweighted pair group method with arithmetic mean (UPGMA), The dissimilarity distance between the operational taxonomic units (OTUs) was calculated using the average Euclidean distance coefficient for mixed data set **(Sneath and Sokal, 1973).**

2.8. Bacterial identification

The promising actinomycetes' isolate was cultured in starch-nitrate liquid medium for seven days and genomic DNAs were extracted with the genomic DNA extraction protocol of Gene Jet genomic DNA purification Kit (Fermentas).Polymerase chain reaction (PCR) using Maxima Hot Start PCR Master Mix (Fermentas).The amplifications were carried out in a thermal cycler (Multigene Optimax, Labnet international, Inc). The PCR thermocycler was programmed as follow: 95°C for 5 min for initial denaturation, 30 cycles at 95°C for 1 min, 55 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 10min. The PCR mixture contained 25 pmol of each primer, 10 ngchromosomal DNA, 200 mmol/LdNTPs and 2.5 U of *Taq* Polymerase in 50 µL of Taq polymerase buffer 10X Standard Taq Reaction Buffer.

The PCR Clean-Up of the PCR product was performed by using Gene JET™ PCR Purification Kit (Fermentas) at Sigma Scientific Services Company, Egypt, 2013. The sequencing of the PCR product was made by the GATC Company by using ABI 3730xl DNA sequencer with universal primers (16S 27F and 16S 1492R), (5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-GGTTACCTTGTTACGACTT-3′). Genotypic characterization was performed using 16S sequence analysis. Multiple alignments with sequences of the most closely related members and calculations of levels of sequence similarity were carried out using BioEdit (software version 7). **(Hall, 1999)** Sequences of rRNA genes, for comparison, were obtained from the National Center for Biotechnology Information (NCBI) database.

3. RESULTS AND DISCUSSION

3.1. Seasonal/spatial distribition of marine actinomycetes

To evaluate the shore of Suez Bay, Egypt, as a source of actinomycetes, ten sampling sites were selected to represent different ecosystems (Figure 1). The entrance channel is affected by the passage of ships to and from the channel. El-Kornesh (It is semi-closed area with low wave motion and broad tide) it is characterized by tourist activities, small fishing boats. Marine high school is affected by human activates, El-zitiat is characterized by arrival and departure of ships, specialized for petroleum oil transportation. On the other hand, sites at Kabanon, NIOF and Attaka are subjected to sewage pollution. Kabanon is affected by the domestic effluent from the old sewage plant of the Suez city which is characterized by muddy sediments with unfavorable habitat for marine life. NIOF is affected by the new sewage treatment plant of the Suez city and near of the wastes of the vegetable oil company. Attaka is affected by barking and repairing the ships in addition to residues of five stars mills factory. Electric power station and Ayoun mossa is affected by waste water from thermal station. Adabyia is affected by the shipping and trading in the harbour (**Hamed, 2013)**.

The average counts of actinomycetes were estimated seasonally in sediment samples from Suez Bay, Egypt, during 2012 (Table 1). In general the summer samples exhibited more actinomycetes counts 22 CFU g^{-1} compared to autumn 15 CFU g^{-1}, spring 14 CFU g^{-1} and winter 8 CFU g^{-1}. The highest counts of actinomycetes in sediment samples recorded in NIOF site while the annual average was 31 CFU g^{-1} flowed by Marin High School (24 CFU g^{-1}), and Electric Power Station (24 CFU g^{-1}). On the other hand the lowest count recorded in Ayoun mossa (2 CFU g^{-1}). Low counts of actinomycetes in sediment of Ayoun mossa site may be due to its far from the pollution sites, while Marine high school and NIOF sites were relative near to the sewage and Suez Steel Company pollution.

Table 1: Seasonal and spatial variation in actinomycetes viable counts (CFU g^{-1}) of Suez Bay sediments during 2012

Sampling site	Season				Annual average
	Winter	Spring	Summer	Autumn	
The entrance channel	15	23	30	18	22
El-kornesh	6	10	15	9	10
Marine high school	0	20	45	30	24
El zitiat	3	3	5	7	5
Kabanon	12	3	15	8	10
Electric Power station	27	24	30	15	24
NIOF	6	35	45	38	31
Attaka	6	10	15	12	11
Adabiya	0	8	15	14	11
Ayoun mossa	0	0	5	3	2

The variations in the environmental factors (temperature, pH, salinity, organic content, and nutrients) of the sediments (Table 2) led to variations in the density of marine actinomycetes. Multiple regression analysis was applied to reflect the relationship between the actinomycetes counts and the environmental factors of sediments during the different seasons.

The regression equation of sediments is: Actinomycetes= -1372.94 – 0.68 pH + 16.73 Temperature – 0.09 phosphate - 11.90 Nitrite + 1.19 Nitrate + 0.43 organic matter- 25.91 Ammonia + 23.09 salinity.

These results show that, the counts of actinomycetes are positively correlated with temperature, dissolved nitrates, dissolved organic matter and salinity and negatively correlated with pH, dissolved phosphate, dissolved nitrite and dissolved ammonia.

The best marine source of actinomycetes identified so far are sediments, from which their isolation is well documented **(Abou-elela, 1999).** The number and variety of actinomycetes present in any sample would be significantly influenced by geographical location, temperature; pH, organic matter content, agricultural activities, aeration, nutrient availability and moisture content **(Isik et al**., **2014).** Variations in the organic content, nutrients and the pollution levels in the different location along Suez Bay, Egypt, led to variations in the density of marine actinomycetes**.**

Table 2: Average Values of physico-chemical parameters in sediment samples during 2012.

Season	physico-chemical parameters							
	pH	Temperature (°C)	Salinity (‰)	Dissolved phosphate (mg at.PO_4-P.l^{-1})	Dissolved nitrate (mg at.NO_3- N l^{-1})	Dissolved nitrite (mg at. NO_2-N l^{-1})	Ammonia (mg at. NH^3-N l^{-1})	Organic matter ($mg1^{-1}$)
Winter	8.23	19.02	41.93	23.29	25.92	0.05	0.40	31.58
Spring	8.49	24.62	41.89	26.96	34.11	0.07	0.18	22.41
Summer	9.07	27.57	41.91	39.49	34.36	0.12	0.29	31.80
Autumn	8.51	22.09	42.51	26.19	40.17	0.09	0.14	24.50

*Data are given per g of sediment
**Average values from different sampling sites (N=10)

3.2. Phenotypic characterization and clustering of actinomycetes

The definition of numerical taxonomy is expressed to apply various mathematical methods for data which are coded numerically and expressed as individual characters and assigning organisms into cluster based on their comprehensive similarities, afterwards presenting relationships in the form of dendrograms. This classification is used by many researchers for bacterial systematic **(Sackin and Jones, 1993).** Thus, numerical taxonomy provides us with an invaluable framework for *Streptomyces* taxonomy, including identification of species.

The starch nitrate medium was the most suitable medium for isolation of actinomycetes as reported by **Ghanem et al. (2000)** and accordingly, it is chosen as isolation medium in this study. Based on the results, all isolates grow well on media prepared without sea water i.e. they are soil inhabitants and well adapted and functional members of the marine microbial community, they are facultative marine and possibly of terrestrial origin. **Goodfellow and Haynes (1984)** reported that, actinomycetes are best known as soil bacteria and were generally believed to occur in the ocean largely as dormant spores that were washed into the sea

A total of seventeen actinomycetes 1isolates (isolated from different locations in Suez Bay sediment) were selected randomly to represent all colony morphologies observed on the starch nitrate agar plates. Pure isolates were then subjected to morphological, physiological and biochemical characterization. The characteristic features of each actinomycetes isolate and the percentage of positive results were described in Table 3. Analysis of the results by numerical techniques using the simple matching coefficient (S_SM) and UPGMA yielded the dendogram in Figure 2. The data show that at 83 % similarity level, the majority of isolates were grouped into two main clusters, cluster A (3 isolates) and cluster B (11 isolates), in addition to single clustered isolates (3 isolates). Cluster A**:** this cluster comprised only three isolates (isolate code: 8, 9 and 14) clustered at 83 % similarity level. Cluster B**:** this group composed 11 isolates (isolate code: 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 17) clustered at 88 % similarity level, and the single clustered isolates (S)**:** three isolates (isolate code: 1, 15 and 16) each of them represented single cluster.

The numerical classification of the genus *Streptomyces* by **Kampfer et al. (1991)** involves determination of 329 physiological tests. The study includes 15 major clusters, 34 minor clusters and 40 single member clusters which were defined at 81.5 % similarity level.

Table 3: The characteristic features (Morphology, physiology and biochemical characterization) of the isolated actinomycetes and the percentages of positive results.

Clusters	**Cluster A**			**Positive %**	**Cluster B**											**Positive%**	**Single cluster (S)**		
Isolates code	**8**	**9**	**14**		**2**	**3**	**4**	**5**	**6**	**7**	**10**	**11**	**12**	**13**	**17**		**1**	**15**	**16**
Growth on																			
-Glucose yeast extract malt extract agar	+	+	+	100%	+	+	+	+	+	+	+	-	+	+	+	91%	-	-	-
-Inorganic salts starch agar	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	-	-
-Czapex Dox agar	-	-	-	0%	-	+	-	+	-	-	-	-	-	-	-	18%	-	-	-
-Krassilnikov agar	+	+	+	100%	+	+	+	+	-	+	+	-	+	+	+	82%	-	-	-
-Oat meal agar	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
- Starch nitrate agar	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
Substrate mycelium																			
Yellow	+	-	-	33%	-	-	+	-	-	-	-	-	-	-	-	9%	-	-	-
Cream	-	-	+	33%	-	-	-	-	-	-	-	-	-	+	-	9%	-	-	+
Pink	-	+	-	33%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
Beije	-	-	-	0%	+	-	-	-	-	-	+	+	+	-	-	36%	-	-	-
Gray	-	-	-	0%	-	+	-	+	+	+	-	-	-	-	+	45%	-	-	-
Pale brown	-	-	-	0%	-	-	-	-	-	-	-	-	-	-	-	0%	+	+	-
Aerial mycelium																			
White	-	-	-	0%	+	-	-	-	-	-	-	+	+	+	+	45%	-	-	-
Cream	-	-	-	0%	-		+	-	-	-	-	-	-	-	-	9%	+	-	-
Gray	-	+	-	33%	-	+	-	+	+	+	-	-	-	-	+	45%	-	-	+
Yellow	-	-	+	33%	-	-	-	-	-	-	-	-	-	-	-	0%	-		-
Pink	-	-	-	0%	-	-	-	-	-	-	-	-	-	-	-	0%	-	+	-
Violet	+	-	-	33%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
Diffusible pigments																			
Beige	+	-	-	33%	+	+	+	+	+	+	+	+	+	-	+	91%	-	-	-
Yellow	-	-	+	33%	-	-	-	-	-	-	-	-	-	+	-	9%	+	-	-
Violet	-	+	-	33%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	+
Dark brown	-	-	-	33%	-	-	-	-	-	-	-	-	-	-	-	0%	-	+	-
Growth at (°C)																			
25-30	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
40	-	+	+	66%	+	-	+	+	-	+	+	+	+	+	+	81%	-	+	-
50	-	-	-	0%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
Growth at pH																			
5-6	-	-	-	0%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
7	+	+	+	100%	+	-	-	+	+	+	+	+	+	+	+	82%	+	+	+
8	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
9	+	+	+	100%	-	-	-	+	+	+	+	+	+	+	+	72%	+	+	+
Utilization of																			
Starch	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
Lactose	-	+	+	66%	+	+	+	+	+	+	+	-	+	+	+	91%	-	-	-
Dextrose	+	+	+	100%	+	+	+	+	-	+	+	-	+	+	+	82%	-	-	-
Maltose	-	+	-	33%	+	+	+	+	-	+	+	+	+	+	+	91%	-	+	+
D-glucose	+	+	+	100%	+	+	+	+	-	+	+	+	+	+	+	91%	+	-	+
Glycerol	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
Growth in presence of NaCl																			
0-4 %	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
7 %	+	-	-	33%	-	-	-	-	-	-	-	-	-	+	+	18%	-	-	-
10 %	+	-	-	33%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
Biochemical tests																			
Protease	-	+	-	33%	-	-	+	+	-	-	+	+	-	+	-	45%	-	-	-

Lipase	-	-	+	33%	+	+	-	+	-	+	+	-	+	-	+	63%	-	+	-
Urease	-	-	-	0%	-	+	-	+	-	+	-	+	+	-	+	54%	-	-	-
Catalase	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	-	+	+
Chitinase	-	-	-	0%	+	-	-	-	-	-	-	-	-	-	-	9%	-	-	-
Oxidase	-	-	-	0%	+	-	-	-	-	-	-	-	-	-	-	9%	-	-	-
Sulphide production	-	-	-	0%	+	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
Melanin production	-	-	-	0%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
Degradation of																			
Cellulose	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	+	100%	+	+	+
Gelatin	+	-	-	33%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
Antibiosis against																			
S. aureus	-	+	+	66%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
E. coli	-	+	+	66%	-	-	-	-	-	-	-	-	-	-	-	0%	-	-	-
V. damsela	-	+	-	33%	-	-	-	+	-	-	-	-	-	-	-	9%	-	-	-
P. aeruginosa	+	+	+	100%	-	-	-	+	-	+	-	-	+	-	-	18%	-	-	-
A. hydrophila.	-	-	+	33%	-	-	-	+	+	+	-	-	-	-	-	27%	+	-	-

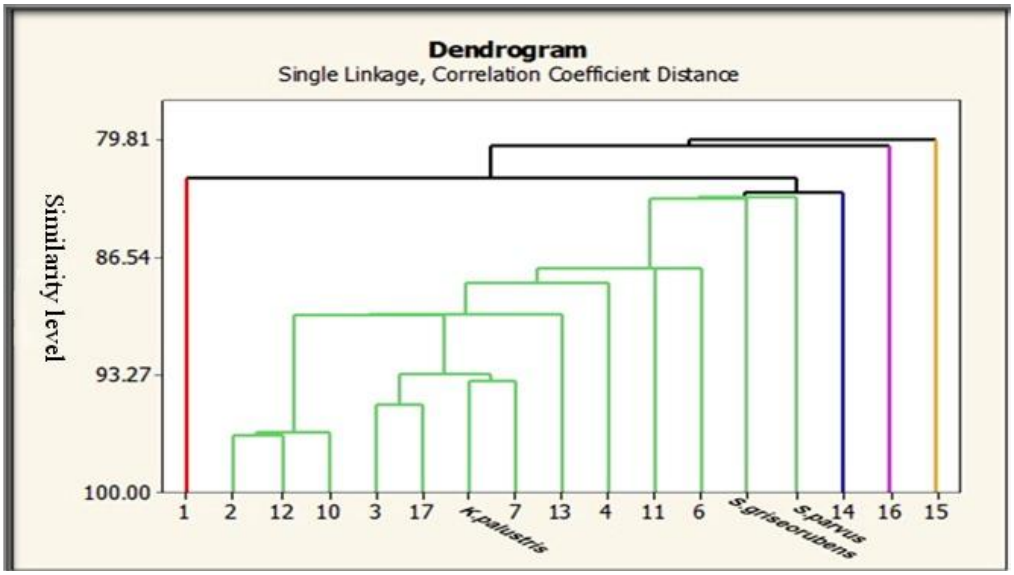

Figure 2: Simplified dendogram showing the relation clusters based on the S_{SM}-UPGMA analysis

3.3. Bioactivity of the isolates

All actinomycetes isolates (17 isolates) were subjected to primary screening of their antagonistic effect against five references bacterial pathogens. Seven isolates showed antagonistic effect at least against one pathogens, etc. 41% of actinomycetes isolates were able to produce antibacterial agents. The most active isolates were isolates number 5, 8 and 9, they exhibited the highest inhibition zones and inhibited 60, 80 and 80%, respectively, of the tested bacterial pathogens. Figure 3 showed the inhibition zones of the most active isolate.

In previous studies, the bacterial antagonism percentage was lower (5 - 8 %) **(Nair and Simidu, 1987)** or higher (35-54 %) **(Long and Azam, 2001). Patil et al. (2001)** mentioned that, actinomycetes were isolated from Tuticorin coast (India). Seventy seven out of one hundred and four isolates were able to inhibite at last one of the pathogens *viz. Aeromonas hydrophila*, *A. sobria* and *Edwardsiella tarda*. The highest antagonistic activities were detected by the isolates form sediment samples and they identified as *streptomyces*. The antagonistic marine *streptomyces* isolates or the antibacterial substances produced by them could be used as antibiotics, which might have a future application in aquaculture system.

(A) (B) (C)

Figure 3: Antagonistic effects of isolate 8 against (A): *Aeromonas hydrophila*, (B): *Pseudomonas aeruginosa* and (C): *Escherichia coli*.

3.4. Molecular phylogeny of the selected isolates

The identification of actinomycetes depends on three axis, the morphological and physiological characterization, the numerical identification and the phylogenetic characterization. Phylogenetic analysis based on 16S rDNA sequence comparison for establishing phylogenetic and evolutionary relationships among organisms **(Isik et al., 2014; Sharma, 2014)**.

Based on results of clustering, numerical taxonomic analysis and antagonistic interactions, three promising actinomycetes isolates produced antimicrobial agents capable of inhibiting the growth of most tested bacterial pathogens, they were selected for identification and molecular phylogenetic analysis. These isolates were 5, 8 and 9. Genomic DNA of each actinomycetes isolates was prepared, and the gen coding for 16S rRNA was partially amplified using the universal primers. The produced amplicons of the selected isolates were detected using agarose gel electrophoresis as shown in Figure 4.

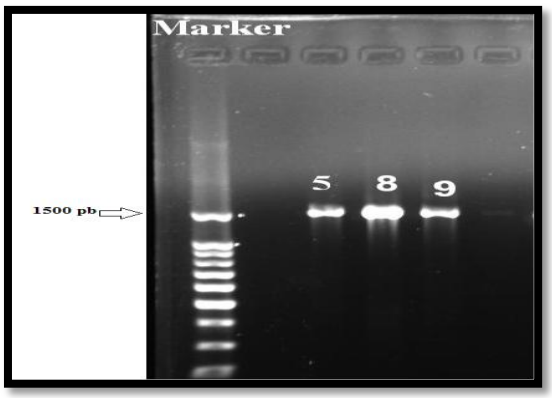

Figure 4: Agarose gel electrophoreses of the amplified 16S rRNA gene of the isolates 5, 8 and 9

The sequences compared with those which gave the highest homology based on a NCBI Blast search. The resulting data indicated that, the isolates under study were similar to species *Kocuria palustris* strain Abk-6, *Streptomyces parvus* strain NRRL-B-1455 and *Streptomyces griseorubens* strain NBRC12780 with similarity 95 %, 96 % and 95 %, respectively. The nucleotide sequences for closely related species were deposited to Gen Bank sequence data base and have KP280002, KP675949 and KR133201, respectively, accession numbers (Table 4). The phylogenetic relationship for this experimental isolates and the closely related relatives were analyzed as shown in Figure 5. Based on these results, the experimental marine actinomycetes have been identified in this work as *Kocuria palustris* (isolate 5, cluster B), *Streptomyces parvus* (isolate 8, cluster A) and *Streptomyces griseorubens* (isolate 9, cluster A).

Table 4: Accession numbers of experimental 16S rRNA sequences and their similarity percentages to the closest known species.

Isolate code	Identification	Accession no.	Similarity %
5	*Kocuria palustris*	KP280002	95
8	*Streptomyces parvus*	KP675949	96
9	*Streptomyces griseorubens*	KR133201	95

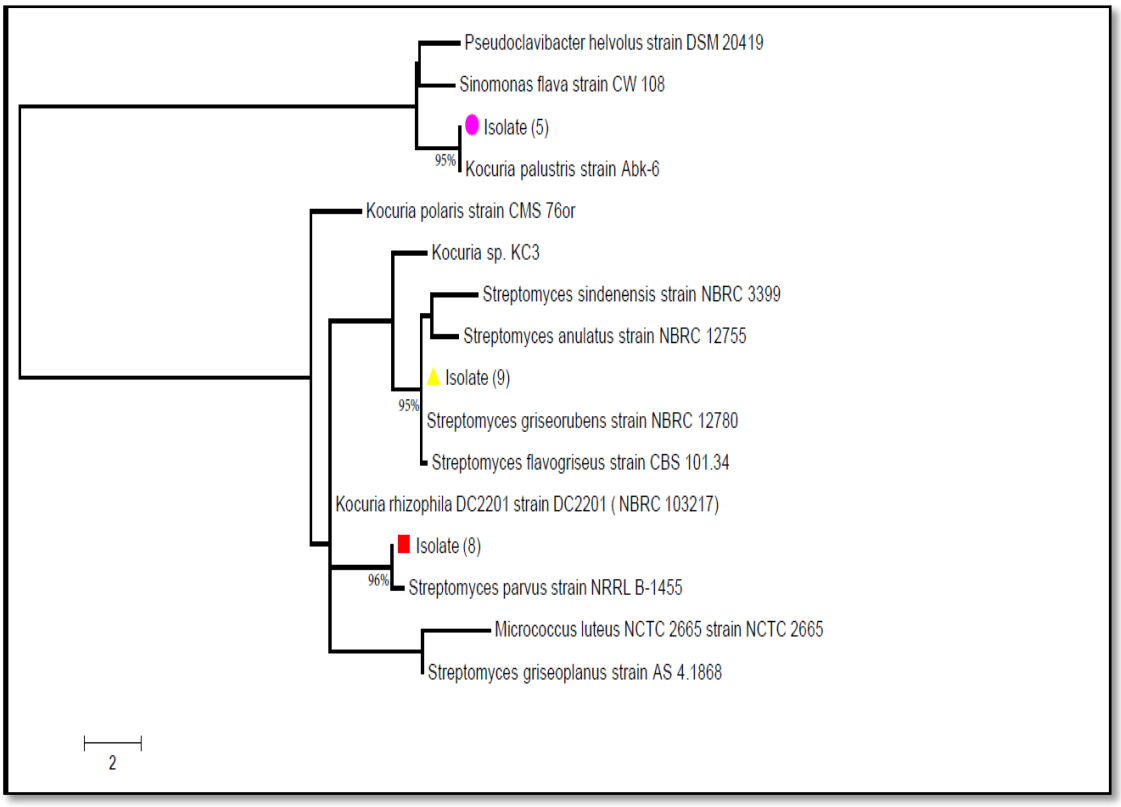

Figure 5: Phylogenetic tree of isolates 5, 8 and 9. 16S rDNA-based dendogram showing the phylogenetic position of isolates among representatives of related bacterial species..

The genus *Streptomyces* seems to provide wide variety of new antibiotics more than any other genus; hence, it is of the most importance for both industrial application and human health care **(Sajid et al., 2011)**. They produce a large number of secondary metabolites and particularly antibiotics that are in favor of pharmaceutical companies nowadays, resulting in conduction of widespread investigation towards discovery of new antibiotics. **(Worrall and Vijgenboom, 2010)**. They were distinguished as Gram-positive bacteria with a high GC content in their DNA to more than 70 % **(Ventura et al., 2007)**.

In addition, *Kocuria palustris* produced an antagonistic agent of broad spectrum, effective against *Vibrio damsela*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila*. The genus *Kocuria* was divided from the genus *Micrococcus* on the basis of the phylogenetic and chemotaxonomic **(Reddy et al., 2003)**.

4. CONCLUSION

Marine environment is a good source for valued species that need to be explored. Results confirmed the richness of the local coastal sediment of Suez Bay, Egypt, with active antibacterial compounds- producing actinomycetes. In conclusion, preliminary isolation studies shows that the strains from the genera *Streptomyces* and *Kocuria* isolated from Suez Bay sediment, obtained in this work might have great capacity for bioactive secondary metabolites such as antibiotics. In order to reveal biotechnological capabilities and pharmaceutical applications of these strains, further molecular and chemotaxonomic studies will be carried out.

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