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SCREENING OF MARINE ACTINOMYCETES FOR THEIR ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES IN EGYPT

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ABSTRACT

The genus *Streptomyces* is represented in nature by the largest number of species and varieties among the family Actinomycetaceae. This study aims to isolate and characterize novel strains of Streptomyces with high antimicrobial and antifungal production capability. The morphological , biochemical and biological characteristics of marine actinomycetes isolated from the coast of the red sea of Seini , Egypt, are presented. A total of 50actinomycetes isolates were isolated by serial dilution plate technique, of these 20 isolates showed activity in primary screening against test pathogens used in this study.Antimicrobial activity of cultivation medium was determined using the diffusion - disc method. As test microorganisms *Bacillus Subtilis*, *Bacillus cereus*, *Escherichia coli,Pseudomonasaeruginosa*, *Lactobacillus casei*and *Candida albicans,* are applied. The same isolates showed inhibitory effects against pathogenic fungi i.e. *Alternariaalternata, A. solani*, *Colletotrichumgloeosporioides, Fusariumoxysporum, Aspergillusflavus* and*Aspergillusniger*. This may indicate the same compound was responsible for the antibacterial activity of the actinomycetes. Out of 20 isolates, 13 isolates exhibited both antibacterial and antifungal activity.Finally potent actinomycetalisolate were selected and it's tested for enzymes production. The isolates were produced most important hydrolytic enzymes as chitinase and β-1, 3 glucanase , cellulose and protease. The culture filtrate of these strains were effective against Alternariaalternata*, A. solani* and *Colletotrichumgloeosporioides*in tomato under greenhouse conditions.

KEYWORDS: **Antifungal, *antimicrobial* activities, secondary metabolites*,Streptomyces sp.,***

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INTRODUCTION

Marine microorganisms have become an important source of study in the search for novel microbial products (Gulve and Deshmukh 2011).Today both academic and industrial interest in marine microorganisms is on the rise. Marine actinomycetes are useful and suitable source of new bioactive natural products. Streptomyces are a group of prokaryotes that are usually found in all types of ecosystems including water and soil. The genus *Streptomyces* is represented in nature by the largest number of species and varieties, which differ greatly in their morphology, physiology, and biochemical activities. Actinomycetes are widely distributed group of microorganism in nature and have the capacity to synthesis many biologically active secondary metabolites (*Rao et al.* 2013). It is possible to isolate strains which substrates are pollutants which contribute to environmental protection. Primarily, the advantage is a possibility of isolation a strain which metabolite product high value product or finds a new metabolite which leading to the development of new products (Atta and Ahmad 2009 and Selvameenal *et al*., 2009). Actinomycetes have been a source of a numerous useful products including pharmaceuticals, agrochemicals, enzymes for use in a number of industrial applications from food industry to papermaking (Rao *et al.* 2013). The secondary metabolites like antibiotics, herbicides, pesticides, anti-parasitic and enzyme inhibitors are obtained from actinomycetes are of special interest due to its diverse biological activities like antibacterial, antifungal, antioxidant, antitumor and antiviral (Baltz, 2005 and 2006 and Ilic *et al*., 2007). Streptomycetes are found in plant rhizosphere and attention has been paid to the possibility that they can protect roots by inhibiting the development of potential fungal pathogens. This may be achieved through by the production of enzymes, which degrade the fungal cell wall, or antifungal compounds (Errakhi*et al*., 2007).In the present investigation an effort was made to screen antagonistic marine actinomycetes from cost of red sea of Seini ,Egypt which is large diverse and largely unscreened ecosystem for the isolation of potent enzymes producing actinomycets. Several isolates were screened for *in vitro* inhibitory activity against six plant pathogenic fungi. The *in vivo* control efficacy against *Alternariaalternata, A. solani*, and*Colletotrichumgloeosporioides* of tomato seedlings was evaluated.

MATERIALS AND METHODS

Isolation of bacterial strain

The samples were collected from ElAreash coast in red sea Seini, Egypt . Collection of sediment samples Twenty near-sea shore sediment samples were collected, 10 cm in depth in sterile Petri plates of sampling sites. Four sediment samples were collected from each site. Samples were collected from 20 m away from the sea shore. All samples were labeled and were brought to the laboratory. They were stored at a temperature between 4^0C until further use. Five g of sample was transferred into a sterile bottle, then 45 ml of sterile distilled water was added and shaken for 30 min. Five sets of ten-fold serial dilutions were prepared from original supernatant, then 100 µl of third concentration was used for inoculation of Starch Casein Agar (SCA) and the plates incubated for 7 days at 28°C. Morphological features of colonies such as colony pigmentation were used for preliminary classification of the bacterial population.

Genus identification and morphological characteristics

Visual observation of both morphological and microscopic characteristics using light microscopy, acid-fastness and Gram-stain properties were performed. All morphological characters were observed on ISSA and were used for classification and differentiation as follows: Aerial mass color; Substrate mycelium; Melanoid pigments and Spore chain morphology. Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974), and in the category IV of the were used for identification.

Biochemical screening

Biochemical criteria such as the ability to degrade Tyrosine reaction Starch hydrolysis , Casein hydrolysis , Gelatin hydrolysis , Nitrate reduction , Citrate , Urease Catalase as

substrates by the various *Streptomyces* strains were used for genus confirmation (Korn-Wendisch and Kutzner, 1991).

Antimicrobial activity

Antimicrobial activityof the cultivation medium was determined by diffusion - disc method using a steril discs. Steril disks were impregnated with samples of liquid cultures of active strains, dried, and placed onto petri dish, previously seeded with test organisms, microorganisms *Bacillus Subtilis*, *Bacillus cereus*, *Escherichia coli,* , *Pseudomonas aeruginosa*, *Lactobacillus casei*and *Candida albicans*. After incubation at 30° C for 48 hours, the incubation zones were measured .

Assay of Antifungal activity

In vitro antagonistic bioassay

The actinomycete isolates were evaluated by a dual-culture *in vitro* assay for their activity towards six pathogenic fungi: *Alternariaalternata, A. solani*, *Colletotrichumgloeosporioides,Fusariumoxysporum, Aspergillusflavus* and *Aspergillusniger*. These fungi were maintained on potato dextrose agar (PDA) at room temperature. Discs (8 mm) from 5 days old colonies of the test fungi, grown on potato dextrose agar (PDA) at 28ºC, were placed at the center of PDA plates. Two discs of the actinomycete (8 mm) from 5 days old colonies, grown on yeast malt extract agar (YM) at 28ºC, were placed on opposite sides of the plates, 3 cm away from the fungal disc. Plates without the actinomycete disc served as controls. All plates were incubated at 28ºC for 10 days and the incubation zones were measured.All isolates were tested in triplicate.

Screening of actinomycetesfor enzyme production

The potent isolated actinomycetes strains were selected and screened for the presence of different enzymes like chitinase, proteases, cellulases and 1,3-β-glucanase.

Chitinases

a.Primaryscreening for chitinaseproduction

Screening for chitinase production of all theisolates was done by plate agar assay. Thecolloidal chitin medium contained: colloidalchitin-15g; yeast extract-0.5g; (NH4)2SO4-1g;MgSO4 · 6H2O-0.3g; KH2PO4-1.36g; agar-15g,and distilled water- 1000ml. The plateswere incubated for 5 days at 30˚C. Theseisolates produced large clear zones on(colloidal chitin agar) CCA (Gadelhak*et al*., 2005).

b. Chitinase assay

50ml of colloidal chitin broth was incubatedwith inoculum at 30˚C in the incubatorshaker at 150 rpm for 7 days. The cell freesupernatant was collected and the assay wasperformed. Colloidal chitin was used as asubstrate to assay chitinase activity: 0.1 g in1 ml of phosphate buffer (pH 7.0) wasincubated with 0.5 ml of enzyme at 37˚C for60 min. The reducing sugars in the reactionmixture were measured by colorimetricmethod.One unit of enzyme activity was defined asthe amount of enzyme that catalyzed therelease of 1µmol of N-acetyl glucoseaminepermlin 60 min(Gadelhak*et al*., 2005).

ii. Estimation of extracellular Glucanase Activity

Plates with ISP containing (1%, w/v) Carboxy Methyl Cellulose (CMC) were prepared. Strains were spot inoculated in the centre of the plate. After an appropriate incubation period at 28°C, the agar medium was flooded with an aqueous solution of Congo red for 15 min. The Congo red solution was then poured off, and plates containing CMC were visualized for zones of hydrolysis detecting β-1,4glucanase. Azo-glucan containing plates by Chen *etal.,*[1997] was used to detect β-1,3 glucanase. ISP medium with (1%, v/v) azoglucan was prepared and spot inoculated with the isolate. Deposition of blue azodye surrounding the colony indicated enzyme production.

1,3-β-glucanase enzyme assay

***Enzyme activity assay*.**

Unless otherwise stated, the standard activity assay for 1,3-β-glucanase was carried out at 65 °C for 5 min, using 0·5% (w/v) laminarin in 50 mM sodium acetate buffer (pH 5·5). The glucose equivalents released from enzyme reactions were determined colorimetrically by the dinitrosalicylic acid method . One unit of activity is defined as the amount of enzyme required to release 1 µmol glucose equivalents min^{-1} under the reaction conditions described above. Protein concentration was determined by the Coomassie blue method (Bradford,

1976) using bovine serum albumin as the standard.

iii. Proteases

a. Primary screening for protease production

Skim milk agar medium contained: skim milk powder, 10g; peptone, 5g; NaCl, 3%; and agar 20g per 1000ml of distilled water; pH 7.0. The actinomycetes strains were streaked on the medium and incubated at 28˚C for 4 days such that colonies were grown. A clear zone appears around the actinomycete colonies if the strain is positive for protease activity (Mitra andChakrabartty 2005).

b. Protease assay

Proteolytic activity was assayed using caseinas the substrate. A 0.5 ml aliquot of theenzyme extract was incubated with 1 ml of2.0% casein solution in 0.1 M TrisHClbuffer, pH 7.0 at 37˚C for 10 min. Thereaction was stopped by the addition of5.0ml 5% trichloroacetic acid and incubatedfor 30 min. The mixture was filtered and2.0ml of filtrate was added to 4.0ml of 0.1NNaOH and 0.5ml diluted Folin-Cocalteaureagent and incubated for 30 min and thenthe amount of tyrosine released into thefiltrate was measured from its absorbance at670 nm. Protein was estimated using BSA asthe standard (MitraandChakrabartty 2005).One unit of protease activity is expressed as the amount of enzyme which converts 1µg of tyrosine per 1min at 37˚C7

iv. Cellulases

a. Primary screening for cellulose production

The actinomycetes strains were grown onISP-4 media (carboxymethyl cellulose-10g, CaCO3-a pinch, (NH4)2SO4-2g, MnCl2-1mg,ZnSO4-1mg, FeSO4-1mg, agar-20g) for 3-4days and then flooded with 1% 5ml of iodinesolution along with 1ml mercuric iodide.Clear yellow zones were observed and thearea was measured.

b. Cellulase assay

50 ml of ISP-4 broth was taken for eachpositive strain and inoculated with thestrain. The strains were incubated for 48-72hrs and then centrifuged in refrigeratedcentrifuge for 10min at 2,000 rpm. The cellfreesupernatant was collected andenzymatic assay was performed. 0.1ml ofcrude enzyme extract was added to 1 ml ofcarboxymethyl cellulose substrate and madeup to 3ml with distilled water. The mixturewas incubated at 60˚C for 20min and thenreaction was stopped by addition of DNSreagent. The absorbance was measured at540nm (Murugan*et al*., 2007).1 unit (IU) is defined as the amount ofenzyme that released 1µmole of glucose from carboxymethyl cellulose per minute atpH 7.0 at 60˚C.

In greenhouse inhibition of A. alternata, A. solani and C. gloeosporioides mycelia infection of tomato plants

Strains were cultivated in 500 ml of YM broth, pH 7.0 and incubated at 28ºC with shaking at 125 rpm for 10 days**.** Bacterial cells were separated by centrifugation at 11,000 rpm for 15 min, and the supernatant was filtered (Milipore filter, 0.45m). The cell-free filtrate (50 %) was used to evaluate the inhibition of infection by *Alternariaalternata, A. solani* and*Colletotrichumgloeosporioides*in tomato seedlings. The seedling (30 days) of commercial tomato plant were planted in plot sizes of 5□5 m2 and planting distances of 20□20 cm2 with one seedling per planting hole.The plants were separated in three groups. A mycelia suspension of *Alternariaalternata, A. solani* and *Colletotrichumgloeosporioides* was prepared: three fungal discs (8 mm diameter), from 5 days old cultures on potato dextrose agar (PDA) at 28^0C, were inoculated into 500 ml of PDB, and incubated at 28^oC with shaking at 125 rpm for 3 days**.** The mycelia mass was collected by centrifugation at 11,000 rpm for 15 min and washed twice with sterile water to remove residual nutrients, then stirred in 300 ml of sterile water for 2 h. The mycelia fragments were collected by centrifugation at 11,000 rpm for 15 min and re-suspended in sterile water at a concentration of 10^4 fragments/ml. Culture filtrate of strains, or sterile distilled water was sprayed on the plants to wetness. After air-drying, hyphal fragment of the pathogen were sprayed on the same plant again. After 15 days, spots symptoms were evaluated

Disease evaluation

Efficacy of *Streptomyces* wereevaluated based on the percentage of infected plants. The percentage of infected plantswere calculated

based on the proportion of infectedplants. Ten randomly selected plants from eachtreatment were scored for disease severity using the StandardEvaluation System of IRRI (IRRI, 1996) was: 0 = nosymptoms, 1 = 1-5 % infected leaves, 3 = 6-12%, 5 =13-25%, 7 = 26-50% and 9 = 51-100% infected leaves.

Statistical analyses

The effects of the treatments on disease severity were analyzed by using Duncan Multiple Range Test (SPSS software 16).

RESULTS AND DISCUSSION

Isolation of actinomycetes

Actinomycetes (50 isolates) were isolated from the different zones of El Aresh coast in red sea of Seini, Egypt by dilution-plate technique on Starch Casein Agar media. The isolates were sub-cultured to obtain pure cultures and were stored at 40^0C for further use.

Characterization of isolates

The morphological and biochemical characteristics of the promising isolates were represented in Tables 1 and 2. Most of the colonies that grew on media belonged to the genus *Streptomyces* since the colonies were slow growing, aerobic, glabrous or chalky, heaped, folded and with aerial and substrate mycelia of different colors. In addition, all colonies possessed an earthy odour. All *Streptomyces*isolateswere acid-fast negative and Gram-stain positive. Microscopically, it was observed that the morphology of the spore chains varied depending on the species, showing the expected straight or flexuous forms, hooks, open loops and coils, which were used, among other features, to establish differences between them. The production of melanoid pigments was variable in all the series with the exception of the strains grouped in the white series, where none produced melanoid pigments (Table 1).The biochemical characteristics of these isolates were further studied. Various biochemical tests executed for the identification of the potential isolates were Tyrosine reaction, Starch hydrolysis, Casein hydrolysis, Gelatin hydrolysis, Nitrate reduction, and the results are tabulated in table 2. Regarding the utilization of caseine, ureas, gelatin, starch and tyrosine, variable results were found.

Antimicrobial activity

Antimicrobial activity of 20 promising isolates were tested against *B. Subtlis, B. cereus*, *E. coli*, *P. aeruginosa*, *L. casei* and *C. albicans* and among them strain 20 isolates were found to have potential activity against most of the test microorganisms which Zone of inhibition with the largest diameter, was formed after 7 days of cultivation (Table 3). Among these isolates *Streptomyces* 5, 7, 10, 17, and 20 show the highest activities againstall the test organisms. Whereas *Streptomyces* 12,13,19 and 2 has moderate activity against all bacteria.Among all the members of the actinobacteria the *Streptomyces* species were known as the producers; of the secondary metabolites such as antimicrobial compounds and cytotoxic compounds that have the potential to control wide range of pathogens (Atta, Ahmad 2009 and Selvameenal *et al*., 2009).

Antifungal Activity

The result of preliminary screening based on the effect of intact bacteria revealed 20 potent isolates in terms of antifungal activity against indicator fungi i.e. *Alternariaalternata, A. solani*, *Colletotrichumgloeosporioides, Fusariumoxysporum, Aspergillusflavus* and *Aspergillusniger*(Table 4). Out of 20 active isolates 5 isolates (*Streptomyces* 5, 7, 10, 17, and 20) showed best activities against the fungi. The isolates *Streptomyces* 12,13,19 and 2 were also effective in their activities against the pathogenic fungi used in the study.Microbial antagonists are widely used for the biocontrol of fungal plant diseases. Many species of actinomycetes, particularly those belonging to the genus *Streptomyces,* are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi (Errakhi *et al.,*2007). The antagonistic activity of *Streptomyces* to fungal pathogens is usually related to the production of antifungal compounds (Fguira*et al.,*2005) and extracellular hydrolytic enzymes (Mukherjee and Sen 2006). The antifungal potential of extracellular metabolites from *Streptomyces*against some fungi was previously reported (Errakhi*et al.,*2007 and Sousa *et al*., 2008).

Potent isolateswere identified *as : Streptomyces habjioensis*(Streptomyces *1); Streptomycesaureofaciens (*Streptomyces *2); Streptomyces hygroscopicus*(Streptomyces *5);* Streptomyces griseus(Streptomyces *7*) *; Streptomycesnogalator*(Streptomyces *10); Streptomycesolivaceiscleroticus,*(Streptomyces *12); Streptomyces lydicus*(Streptomyces *13);* Streptomyces nodosus(Streptomyces *14); Streptomyces violaceusniger*(Streptomyces *16); Streptomycesantibioticus*(Streptomyces *17); Streptomycesspectabilis*(Streptomyces *18);* Streptomyces*fradiae*(Streptomyces *19) ;* Streptomyces*celluloflavus* (Streptomyces *20).*

Table 1
Morphological characteristics of Streptomyces isolates

Isolates Numbers	Aerial hyphae colour	Vegetative Mycelia colour	Growth	Spore mass colour	Soluble pigment
2	Brown	brown	Abundant	Brown	Brown
2	Grey	Creamy	Abundant	Blue	Reddish brown
3	yellow	yellow	Good	Light yellow	Dark brown
2	Brown	Light brown	Abundant	Light Brown	Dark brown
3	Grey	Green	Abundant	Dark green	Dark green
5	Grey	Creamy	Good	Grey	Brown
3	Brown	Brown	Abundant	Light Brown	brown
2	Brown	Light brown	Good	Light Brown	Dark brown
1	Green	White	Abundant	White	Green
2	Grey	White	Good	White	Creamy
3	Brown	Light brown	Abundant	Light Brown	Brown
3	Light Brown	Light brown	Abundant	Light Brown	Brown
3	Light Brown	Light brown	Abundant	Light Black	yellow
3	Light Green	Creamy	Abundant	Green	Creamy
2	brown	grey	Abundant	grey	brown
2	yellow	Light brown	Abundant	red	Creamy
2	Grey	yellow	Abundant	grey	Grey
3	brown	Light brown	good	grey	yellow
4	Creamy	yellow	good	grey	Creamy

Table 2
Biochemical characteristics of Streptomyces isolates

Isolates Numbers	Catalase	Urease	Citrate	Nitrate reduction	Gelatin hydrolysis	Casein hydrolysis	Starch hydrolysis	Tyrosine reaction
2	-	+	+	+	+	-	-	+
2	+	+	-	+	+	+	+	+
2	-	+	+	+	+	+	+	+
2	-	+	+	+	-	-	-	+
3	-	+	+	+	+	-	+	+
5	+	-	+	-	+	-	+	-
3	+	+	+	+	+	+	+	+
3	-	+	+	+	+	+	+	+
1		-	+	-	-	-	-	+
2	+	+	+	+	+	+	+	+
3	-	+	+	+	+	-	-	+
3	-	+	+	+	+	-	-	+
3	-	+	+	-	+	+	+	+
3	+	+	+	+	-	+	-	+
2	-	+	-	+	-	+	-	+
2	+	+	+	+	+	-	+	+
2	-	-	+	+	+	+	+	-
3	+	+	-	+	-	+	-	-
4	+	+	+	-	+	+	+	+
2	-	+	+	+	-	+	+	+

Enzymatic activity of actinomycetes

Enzymatic activities of 13 potent actinomycetal isolates were performed for possessing chitinase, glucose, protease and cellulose activities (Table 5) .*Streptomyces hygroscopicus*and *Streptomyces griseus*, were found to be positive for chitinase production using colloidal chitin agar media. In chitinase assay, NAG (N-acetyl glucose amine) was used as standard. Strain *Streptomyces hygroscopicus* has exhibited optimum activity of 6.231µmol/ml/hr when ISP-2 media was used and incubated till day 7 at 37^0C. For glucanase screening, the isolates were grown on *Streptomyces* 20, 17, 14 and 13 agar plates for 4-5 days and then were tested for glucanse production. A clear white zone is formed surrounded by violet background. Strain *Streptomyces hygroscopicus* exhibited optimum activity of 7.75 µmol/ml/min on day 3 at 370C and pH 7.0.In protease assay tyrosine was used as standard. Strain *Streptomyces nogalator* showed enzymatic activity of 0.792 µmol/ml/min on day 4 at pH 8.0 and temperature 370C.For cellulase screening, the isolates were grown on ISP-4 modified agar media where cellulose or CMC was used instead of starch. After 4 to 5 days incubation, the plates were flooded with 5ml of iodine solution along with 1ml mercuric iodide. Here pale yellow color zones were formed indicating cellulase production. Strain *S. celluloflavus* and *Streptomyces lydicus* were found to be positive. For cellulase assay, glucose (0.1mg/ml) was used as standard. DNS method was used to measure absorbance at 540nm. Strain *S. celluloflavus* has exhibited an optimum activity of 10.475 µmol/ml/min on day 5 at 370C and pHChitinase and β-1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls, as for example, cell walls of *Fusariumoxysporum, Sclerotinia minor*, and *S. rolfsii* (El-Katatny*et al.,* 2001).*In greenhouse* inhibition of *A.* alternata*, A.* solani *and C*. gloeosporioides mycelia infection of tomato plants.It was also observed that, all the selected bacterial isolates were effective in controlling fungal pathogens significantly in tomato plants grown under greenhouse conditions (Table 6) . The seedlings treatment with co-inoculation of the pathogens showed reduction in diseases by the treatments as compared to the seed treated with pathogens alone. Treated seedling with*Streptomyces hygroscopicus,*Streptomyces griseus*, Streptomyces olivaceiscleroticus, Streptomyces aureofaciens , Streptomyces nodosus*and *Streptomyces antibioticus* showed completely control of tomato phytopathogens*A. alternata, A. solani* and *C. gloeosporioides*.

Table 3
Antibacterial activity of the Streptomyces isolates

Strains	Antimicrobial activityInhibition zone diameter in mm					
	Bacillus Subtilis	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Lactobacillus casei*	*Candida albicans*
***Streptomyces* 1**	15.4	18.7	15.2	12.8	12.7	10.9
***Streptomyces* 2**	17.7	21.7	17.2	13.6	15.0	12.3
***Streptomyces* 3**	13.2	16.8	14.3	12.8	12.0	9.4
***Streptomyces* 4**	14.2	14.7	13.5	13.8	12.1	13.9
***Streptomyces* 5**	19.8	26.7	18.8	17.6	19.1	18.4
Streptomyces 6	14.0	16.8	15.4	16.7	15.5	11.8
***Streptomyces* 7**	26.4	22.5	20.2	18.6	18.8	17.8
***Streptomyces* 8**	14.4	14.6	12.4	11.8	12.8	10.0
***Streptomyces* 9**	13.4	14.6	11.3	12.6	11.0	10.7
***Streptomyces* 10**	22.2	24.8	18.3	14.2	16.0	15.3
***Streptomyces* 11**	15.6	15.8	14.5	13.8	13.8	11.8
***Streptomyces* 12**	17.7	21.9	15.7	15.4	15	14.8
***Streptomyces* 13**	17.7	17.8	15.5	14.7	15	15.6
***Streptomyces* 14**	18.7	22.4	17.2	16.1	17	14.5
***Streptomyces* 15**	13.4	14.4	12.4	13.4	13.4	13.4
***Streptomyces* 16**	16.4	18.5	15.7	13.4	16.1	12.7
***Streptomyces* 17**	21.6	20.8	18.1	15.2	18	15.9
***Streptomyces* 18**	18.6	19.5	17.2	19.7	21.0	17.2
***Streptomyces* 19**	18.8	16.8	16.7	15.8	19.8	15.9
***Streptomyces* 20**	19.3	20.8	18.1	21.9	18.8	16.5
LSD	1.8	2.1	1.9	2.2	1.9	1.8

Table 4
Antifungal activity of the Streptomyces isolates

Streptomyces isolates	Antifungal activityInhibition zone diameter in mm)					
	Alternaria alternata	*A. solani*	*Colletotrichum gloeosporioides*	*Fusarium oxysporum,*	*A. flavus*	*Aspergillus niger*
***Streptomyces* 1**	14.4	13.6	12.6	11.8	12.8	11.0
***Streptomyces* 2**	15.7	16.5	16.7	18.9	14.6	15.3
***Streptomyces* 3**	13.0	16.0	14.3	12.0	11.0	10.4
***Streptomyces* 4**	13.4	13.4	12.4	13.4	12.4	11.4
***Streptomyces* 5**	17.8	19.6	20.8	21.8	18.8	19.4
Streptomyces 6	14.6	14.8	14.5	12.8	11.8	11.8
***Streptomyces* 7**	21.4	22.5	23.7	25.8	20.8	21.9
***Streptomyces* 8**	13.4	12.6	11.0	11.8	11.8	10.0
***Streptomyces* 9**	12.4	13.6	11.3	11.6	11.0	11.7
***Streptomyces* 10**	19.8	21.7	20.7	19.6	16.7	17.3
***Streptomyces* 11**	13.4	13.6	12.0	13.8	13.8	12.0
***Streptomyces* 12**	16.6	18.7	15.5	15.7	14.4	16.8
***Streptomyces* 13**	15.7	16.9	16.7	18.7	15.3	17.3
***Streptomyces* 14**	17.5	18.9	17.8	18.8	15.7	16.7
***Streptomyces* 15**	13.3	12.3	14.3	14.3	13.3	12.5
***Streptomyces* 16**	15.7	16.5	16.7	15.8	14.4	15.7
***Streptomyces* 17**	18.3	19.7	19.6	20.7	16.7	18.9
***Streptomyces* 18**	17.6	18.5	18.8	22.8	20.8	19.8
***Streptomyces* 19**	11.6	12.0	11.5	12.8	12.8	12.8
***Streptomyces* 20**	18.6	19.1	19.1	19.6	18.8	18.5
LSD	1.5	1.8	2.0	1.9	1.8	1.8

Table 5
Enzymatic activity of Streptomycesactive isolates

	Enzymatic activityμmol/ml/min			
***Streptomyces* isolates**	**Chitinase**	**Glucanase**	**Protease**	**Cellulase**
Streptomyces habjioensis	1.431	2.65	0.132	3.765
Streptomyces aureofaciens	4.420	4.87	0.248	0.132
Streptomyces hygroscopicus	6.027	7.75	0.433	1.388
Streptomyces griseus	6.231	6.86	0.631	3.541
Streptomyces nogalator	5.356	5.87	0.792	2.412
Streptomyces olivaceiscleroticus	5.061	9.543	0.310	2.012
Streptomyces lydicus	4.534	7.654	0.412	9.543
Streptomyces nodosus	4.761	6.012	0.12	4.430
Streptomyces violaceusniger	4.871	2.98	0.320	3.543
Streptomyces antibioticus	5.512	5.54	0.632	4.541
Streptomyces spectabilis	4.843	7.76	0.361	3.760
Streptomyces fradiae	3.512	5.54	0.597	2.132
Streptomyces celluloflavus	3.897	12.56	0.765	10.475
LSD	0.98	1.32	0.24	0.15

Table 6
Antifungal activity of the isolated actinomycetes against Alternariaalternata, A. solani, andColletotrichumgloeosporioides of tomato under greenhouse conditions.

***Streptomyces*Isolates**	Tomato diseases incidence %*		
	A. alternata	***A. solani***	***C. gloeosporioides***
Streptomyces habjioensis	4.5	1.4	0.7
Streptomyces aureofaciens	0.0	0.0	0.0
Streptomyces hygroscopicus	0.0	0.0	0.0
Streptomyces griseus	0.0	0.0	0.0
Streptomyces nogalator	4.6	6.2	5.5
Streptomyces olivaceiscleroticus	0.0	0.0	0.0
Streptomyces lydicus	0.8	0.65	0.0
Streptomyces nodosus	0.0	0.0	0.0
Streptomyces violaceusniger	3.2	3.5	1.3
Streptomyces antibioticus	0.0	0.0	0.0
Streptomyces spectabilis	0.5	0.7	0.7
Streptomyces fradiae	3.7	3.5	2.4
Streptomyces celluloflavus	2.6	2 .9	0.7
Infected control	33.7	41.1	23.1
Untreated control	0.0	0.0	0.0
LSD	0.8	0.34	0.63

The percentage of infected plantswere calculated based on the proportion of infectedplants. Ten randomly selectedplants from eachtreatment were scored for disease severity using the StandardEvaluation System of IRRI (IRRI, 1996) was: 0 = nosymptoms, 1 = 1-5 % infected leaves, 3 = 6-12%, 5 =13-25%, 7 = 26-50% and 9 = 51-100% infected leaves.

In this study, we tried to identify the active strains of *Streptomyces* isolates with high yield of antibiotic and enzymes production from various samples of marine in Egypt using various morphological, biochemical techniques and biological activities as antimicrobial and antifungal . Among all the active isolates, 20 isolates exhibited promising activities against the test organisms (*B. subtilis*, *B. cereus*, *E. coli*,*P. aeruginosa*, *L. casei*and *C. albicans)* used in the study which exhibit antibacterial activity and antifungal against *A. alternata, A. solani*, *C. gloeosporioides, F. oxysporum, A. flavus* and *A. niger*.In this research, the isolation of a *Streptomyces* isolates and its ability to produce extracellular antifungal metabolite(s) against pathogens has been investigated.The isolates produced most important hydrolytic enzymes- chitinase and β-1, 3 glucanase along with cellulose and protease. Out of 20 isolates, 13 isolates exhibited both antibacterial and antifungal activity. The culture filtrate of these strains were effective against *A. alternata, A. solani* and *Colletotrichumgloeosporioides*in tomato under greenhouse conditions. Therefore, these *Streptomyces* can be considered for isolation of novel secondary metabolites which may be of importance for various biocontrol and applications.Actinomyceteshave a profound role in the marine environment apart from antibiotic production (Das *et al.* 2006). The degradation of various materials is a continuous process mediated by the action of a variety of microorganisms. There is a speculation that the increase or decrease of a particular enzyme-producingmicroorganism may indicate the concentration of natural substrate and conditions of the environment (Ramesh and Mathivanan 2009). The antifungal potential of extracellular metabolites produced by soil-borne *Streptomyces* isolates could be exploited for its future use as a biofungicide. The chitinolyticactinomycetes were reportedand various industrially important enzyme producing actinomycetes have been reported (Ramesh and Mathivanan 2009). Streptomycetes have been shown to have the ability to synthesize antibacterial (Ramesh and Mathivanan, 2009), antifungal EbrahimiZarandi*et al.*, 2009), insecticidal (Pimentel-lardo*et al*. 2010), antitumor (Hong *et al.*, 2009), anti-parasitic (Pimentel-Elardo*et al.* 2010), antiviral (Sacramento *et al.* 2004), and herbicidal and plant growth promoting compounds (Sousa *et al*. 2008) as well as many other agents such as enzyme inhibitors (Hong *et al.* 2009) and vitamins (Atta 2007) and hence, they are widely recognized as industrially important microorganisms (Higginbotham and Murphy, 2010). Use of biocontrol agents such as these broad-pectrum*Streptomyces* isolates will probably be one of the important tactics for plant disease management in the near future as they allow the reduced use of pesticides and fertilizers that are potential pollutants of the environment. In conclusion, we identified some of the isolates with high antifungal activity.

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