academicJournals

Vol. 13(34), pp. 3505-3515, 20 August, 2014
DOI: 10.5897/AJB2014.13869
Article Number: 9D61EB746842
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Antimicrobial activity of *Streptomyces* sp. isolated from the gulf of Aqaba-Jordan and screening for NRPS, PKS-I, and PKS-II genes

Fayza Kouadri[1], Amal Al-Aboudi[2] and Hala Khyami-Horani[1*]

[1]Department of Biological Sciences, Faculty of Science, University of Jordan, Amman 11942, Jordan.
[2]Department of Chemistry, Faculty of Science, University of Jordan, Amman 11942, Jordan.

Received 17 April, 2014; Accepted 21 July, 2014

Forty-nine *Streptomyces* isolates were recovered from sediment samples in the gulf of Aqaba/Jordan. All isolates were tested for antimicrobial activity against Gram positive bacteria, Gram negative bacteria, and yeast. Twenty eight *Streptomyces* isolates were active against at least one of the tested strains. The majority of the isolates showed activity against Gram positive bacteria: *Streptomyces aureus* (89%), *Streptomyces epidermidis* (64%) and *Bacillus Subtilis* (50 %). Lower activity was observed toward Gram negative bacteria with only 25% active against *Pseudomonas aeruginosa*, whereas only 17% were active against the yeast *Candida albicans*. Isolate S34 showed best activity. It produced heat stable antimicrobial activity at both acidic and alkaline pH (5 to 5.5 and 8 to 9.5). S34 was found to be related to *Streptomyces rochei*. Forty-nine *Streptomyces* isolates were screened for genes encoding non ribosomal peptide synthetases (NRPS) and polyketides synthases (PKS; types I and II). NRPS sequences were widely distributed and detected in 81% of *Streptomyces* isolates. PKS types I and II were detected in 63.2 and 65.3% of isolates, respectively. Additionally, the relationship between the occurrences of biosynthetic gene sequences (NPRS and PKS sequences) and the production of antimicrobial activities was determined. The above results reveal that the marine *Streptomycetes* are a promising source of novel and unique products.

Key words: Marine *Streptomyces*, antimicrobial activity, non ribosomal peptide synthetases (NRPS), polyketides synthases (PKS), enzymes, gulf of Aqaba, Jordan.

INTRODUCTION

Streptomyces is the largest genus of *Actinobacteria* with over 500 species been reported. Identification of *Streptomyces* and definition of the species is not easy, due to their variety of morphological, physiological and

*Corresponding author. E-mail: horani-h@ju.edu.jo.

Abbreviations: NRPS, Non ribosomal peptide synthetases; **PKS,** polyketides synthases; **A,** Adenylation; **T,** thiolation; **C,** condensation; **ISP,** international *Streptomyces* project.

biochemical characteristics. The methods used for characterization are based largely on morphological observations, subsequent classifications based on numerical taxonomic analyses of standardized sets of phenoltypic characters and, the use of molecular phylogenetic analyses of gene sequences (Labeda et al., 2012). Members of the genus have high Guanine and Cytosine content in their DNA and aerial mycelia (Anderson and Wellington, 2001). They are considered as one of the most important sources of antibiotics (Dharmaraj, 2010; Ayari et al., 2012; Sirisha et al., 2013). They produce about two thirds of the clinically useful antibiotics that are natural in origin (Jensen et al., 2005a) including streptomycin, erythromycin, tetracycline and neomycin. Indeed *Streptomyces* genus in the marine environment is largely unexplored, although true indigenous marine *Streptomyces* species have been described (Bull et al., 2005), suggesting a promising source of novel and unique bioactive metabolites (Maldonado et al., 2005; Moore et al., 2005; Dharmaraj, 2010; Ayari et al., 2012). Increasing number of novel metabolites of commercial interest was isolated from marine *Streptomyces* (Lam, 2006, Wu et al., 2006; Dharmaraj, 2010; Jayaprakashvel, 2012). Potent and diverse bioactivities were reported, they included antibacterial, antifungal, antitumor, and anticancer activities (Newman and Cragg, 2007; Olano et al., 2009).

Large number of bioactive products, with medicinal and agricultural application, are synthesized by non ribosomal peptides synthetases (NRPS) and polyketides synthases (PKS type I and II) (Ayuso-sacido and Genolloud, 2005; Savic and Vasiljevic, 2006). Polyketides synthases are multienzyme complexes that synthesize polyketides by sequential decarboxylative condensation of acyl coenzyme A units (Hopwood, 1997). NRPSs are multifunctional enzyme complexes organized into modules. Each module contains three essential domains: Adenylation (A), thiolation (T), and condensation (C). Evaluation of the biosynthetic potential, expressed in gene detection, has been extensively described in terrestrial *Streptomycetes* (Metsa-Ketela et al., 1999); but very little is known in marine counterparts. The presence of highly conserved sequences in PKSs, and NRPS systems among terrestrial and marine organisms have been used to design PCR primers, targeting ketosynthase (KS) and malonyl transferase in PKS-I, ketoacylsynthase (KS_{α}) in PKS-II and adenylation domains in NRPS (Ayuso-sacido and Genilloud, 2005; Pathom-aree et al., 2006).

Streptomyces have been isolated from different parts of Jordan, including hot spring areas (Abussaud et al., 2013), arid habitats (Saadoun et al., 2008), forest (Saadoun et al., 2007), and soil (Saadoun and Gharaibeh, 2002; Saadoun et al., 1999). Since marine environments, which constitute a rich source of novel and bioactive marine microorganisms is attracting a major focus of many natural products research efforts, and since the Gulf of Aqaba represents the only marine access of Jordan, we chose this site for our study. Gulf of Aqaba environment is unique in terms of its special marine life, represented mostly by intensive coral reef ecosystems and sea grass meadows; it is a narrow deep basin with an average width of 14 km and a total length of 180 km located in the northernmost part of the Red Sea.

As far as we know, this is the first report for the isolation of marine *Streptomyces* from the Gulf of Aqaba, Jordan. Therefore, this study was initiated to evaluate the bioactivity of *Streptomyces* isolates from the Gulf of Aqaba-Jordan; and to screen for the presence of PKS /NRPS genes associated with bioactivity.

MATERIALS AND METHODS

Isolation and characterization of *Streptomyces*

A total of 295 sediment samples were collected from the Gulf of Aqaba. Samples were obtained at different depths (1 to 40 m), they were placed in sterile universal bottles, and immediately processed in the laboratory, according to the following methods (Mincer et al., 2002; Jensen et al., 2005b): Method 1 (dilution), 1 g of wet sediment was added to 4 ml sterile seawater, heated for 6 min at 55°C to reduce non spore forming bacteria. Aliquots of the sample were spread onto the isolation media. Plates were incubated at 30°C for 7 to 45 days. Method 2 (dry / stamp): 1 g of sediment was dried overnight in laminar hood, then ground lightly. Serial dilutions were made by pressing autoclaved foam-plug onto the sediment, then repeatedly onto the surface of isolation media. The plates were incubated at 30°C for 7 to 45 days. Method 3 (dilute / heat): 1 g of dried sediment was added to 3 ml of sterile seawater, then heated to 55°C for 6 min. 50 µl aliquots of the suspension were inoculated onto the isolation media, plates were incubated at 30°C for 7 to 45 days. Method 4 (dry / stamp+ dilute/ heat): tThe dried sediment was processed using method 2, then as in method 3 before inoculation. Plates were incubated at 30°C for 7 to 45 days.

Each sample was incubated into each of four media: Starch-yeast extract agar medium (SYB; Soluble starch 10 g/l, yeast extract 4.0 g/l, peptone 2.0 g/l, agar 18 g/l); Starch- casein agar medium [SCA; Soluble starch 10 g/l, casein (dissolved in 0.3 M NaOH) 1.0 g/l, agar 15 g/l)]; Starch- nitrate broth medium (SNB; Starch 20 g/l, KNO_3 2 g/l, $K_2HPO_4.3H_2O$ 1 g/l, $MgSO_4.7H_2O$ 0.5 g/l, NaCl 0.5 g/l, $CaCO_3$ 3.0 g/l, Trace salt solution 1.0 ml); and Oatmeal agar (OA; Oat meal 20 g/l, trace salt solution 1.0 ml, Agar 20 g/l). Isolation media were supplemented with 100 µg/ml of cycloheximide and 50 µg/ml of nalidixic acid to inhibit the growth of yeasts, fungi and bacteria. All samples were processed in triplicates. Suspected *Streptomyces* colonies were purified on starch casein agar. Pure cultures were maintained on starch casein agar slants at 4°C. They were sub-cultured every three months. For long term storage, isolates were stored in 20% glycerol at -20°C.

Cultural, morphological and physiological characteristics

Isolates were characterized to the genus level according to the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966) and Bergey's manual of Determinative Bacteriology (Buchanan and Gibbons, 2002). For cultural and morphological characteristics of the colonies and the ability to produce soluble pigments, the isolates were inoculated onto the media described by Shirling and Gottlieb (1966), and included inorganic salt-starch

Table 1. Primer sequences used for the detection of NRPS, PKSI, and PKSII genes from *Streptomyces* isolates.

Target Gene	Primer Name	Oligonucleotide sequences (5`-3`)	Product Size(bp)	References
NRPS	A3F	GCSTACSYSATSTACACSTCSGG	700-800	Ayuso-Sacido and Genilloud (2005)
	A7R	SASGTCVCCSGTSCGGTAS		
PKS-I	K1F	TSAAGTCSAACATCGGBCA	1200-1400	Ayuso-Sacido and Genilloud (2005)
	M6R	CGCAGGTTSCSGTACCAGTA		
PKS-II	KS_{α}	TSG CST GCT TGG AYG CSA TC	613	Mesta Ketela et al. (2002)
	KSβ	TGG AAN CCG CCG AAB CCG CT		

agar, oatmeal agar, yeast extract-malt extract agar, and Czapek-Dox agar. The plates were incubated at 30°C in darkness and examined after 7, 14, and 21 days of incubation. The production of melanin pigment, in different media, was determined according to the methods of ISP. The morphology of aerial mycelia was described following Bergey's Manual (Buchanan and Gibbons, 2002).

Carbohydrate utilization was determined by growing isolates on basal mineral salts agar medium supplemented with 1% carbon source at 28°C (Pridham and Gottlieb, 1948; Benedict et al., 1955). Tolerance to NaCl was studied using 4, 7, 10, and 13% NaCl concentration in starch casein agar medium [starch (10 g/l), casein (1 g/l), and agar (15 g/l0].

Screening for antimicrobial activity of *Streptomyces*

Antimicrobial activity was determined using agar well diffusion method (Augustine et al., 2005a). *Streptomyces* isolates were inoculated in starch casein broth medium prepared with 75% seawater. After incubation for 7 days at 30°C with shaking (150 rpm), the supernatants were tested against Gram-positive bacteria: *Bacillus subtilis* ATCC66 33, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* clinical isolate*, Micrococcus luteus* ATCC 10260, *β-hemolytic streptococci* clinical isolate. Gram-negative test strains included: *Escherichia coli* clinical isolate, *Pseudomonas aeruginosa* clinical isolate, *Bordetella bronchiseptica* ATCC 19395, *Klebsiella sp.* clinical isolate, plus the yeast *Candida albicans* ATCC 10231. Antimicrobial activity was expressed as the diameter of the inhibition zones (Laidi et al., 2006). Clinical isolates were obtained from the central laboratory of the ministry of health, Amman, Jordan. Test microorganisms were stored on slants at 4°C, and subcultures monthly. *Streptomyces* isolates (S34) showed the highest activity, and was selected for further studies.

Detection of NRPS, PKS-I, and PKS-П genes

In order to evaluate the biosynthetic potential of bioactive compounds from *Streptomyces* isolates, degenerate primers: A3F/A7R, K1F/M6R and $K_{\alpha}F/K_{\beta}R$ were used (Alpha DNA / Montreal) to detect the presence of NRPS, PSK-I and PKS-II genes in all *Streptomyces* isolates obtained from sediment samples from the Gulf of Aqaba.

DNA extraction

Streptomyces isolates were inoculated in Tryptic Soy broth (Sigma) prepared with 70% seawater, and incubated at 30°C for 48 h with shaking (150 rpm). Genomic DNA was extracted using Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer instructions.

PCR primers

The oligonucleotide primers used for detection of NRPS, PKS-I, and PKS-II NRPS genes were obtained from Alpha DNA (Quebec) (Table 1).

PCR amplification

PCR amplification of NPRS, PKS-I, and PKS-II genes were performed on My Cycler (Bio-Rad, USA) in a final volume reaction of 50 µl, containing 25 µl master mix (Promega, USA), 2 ml of each primer and 5 ml of the extracted DNA. NPRS and PKS-I were amplified with primers A3F/A7R and K1F/M6R, respectively. They were performed as recommended by Ayuso-sacido and Genilloud (2005) and Ayuso et al. (2005) using the following programs: 5 min at 95°C and 35 cycles of denaturizing for 30 s at 95°C, annealing for 2 min at 55°C for K1F/M6R and 59°C for A3F/A7R, and extension for 4 min at 72°C, followed by final extension for 10 min at 72°C whereas, the amplification of PKS-II with primer KS_{α}/KS_{β} was performed using the following temperatures: 2 min at 95°C, 30 cycles of denaturizing of 1 min at 96°C, annealing of 1 min at 64°C, 1.5 min at 73°C and final extension of 8.5 min at 73°C (Pathom-aree et al., 2006).

Gel electrophoresis

PCR products were analyzed using agarose gel electrophoresis by loading 10 µl of each PCR sample and 100 bp DNA Ladder into 1% agarose gels (Promega, USA). The electrophoresis gel was run with 100 V for 1 h, then examined and photographed using gel documentation system.

Identification of *Streptomyces* sp. S34

Isolate S34 was identified according to the description of the *Streptomyces* species recorded in Bergey's Manual and International *Streptomyces* Project (Buchanan and Gibbons, 2002).

Antimicrobial bioassay of isolate S34

Antimicrobial activity of isolate S34 was evaluated in Starch casein broth medium by agar diffusion method against Gram-positive bacteria: *S. aureus* ATCC 6538, Gram-negative bacteria: *E. coli* and

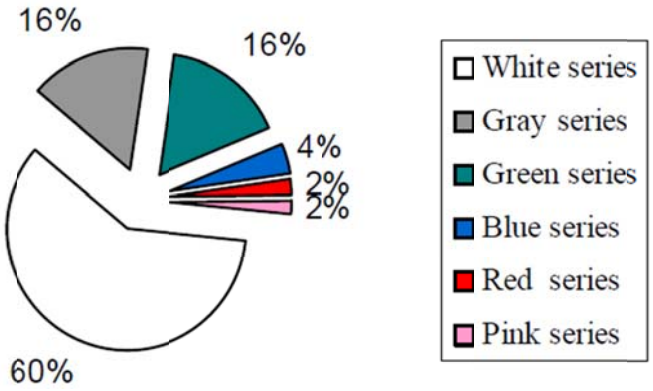


Figure 1. Percentage of *Streptomyces* color series isolated from Aqaba Gulf.

and the yeast *C. albicans* ATCC 10231.

Optimization of antimicrobial compounds production from *Streptomyces* S34

Cell free supernatants of isolate S34 showed significant activity against test microorganisms, thus they were chosen to determine the optimal conditions for bioactivity. Each of the following parameters was optimized: seawater content, effect of medium components, incubation period, pH, temperature, and agitation rate. The bioactivity of S34 was monitored for 14 days (2, 3, 4, 5, 6, 8, 10, 12, and 14 days). The optimal pH and temperature were separately determined by growing the isolate at pH range 3 to 12 with 0.5 differences, and at temperature range of 20 to 50°C with 5°C variance, and agitation rates of 0, 50,100,150, 200 and 250 rpm. All experiments were performed in duplicates. The antimicrobial activity was determined by agar well diffusion assay using nutrient agar medium for *S. aureus* and *E. coli* and Sabouraud agar medium for *C. albicans*.

Thermal stability and the effect of proteolytic enzymes

To study the effect of temperature on the bioactivity of isolate S34, cell free supernatant was heated to 100°C for different time intervals: 5, 15, 30 min, and 1 h. After each interval the supernatant was cooled to room temperature before measuring the residual antimicrobial activity. Supernatants without treatment were used as control (Augustine et al., 2005a). The effect of proteolytic enzymes on the activity was determined by incubating culture supernatant with pepsin and trypsin (Fluka, Germany) at final concentrations of 50 and 100 mg/ml, respectively. Supernatants were then incubated for 1 h at 30°C. The supernatant without any enzyme served as negative control. The residual antimicrobial activity was tested using agar well diffusion assay.

RESULTS

Isolation and characterization of *Streptomyces*

The characterization of *Streptomyces* isolates were performed according to the methods recommended by Bergey's Manual (Buchanan and Gibbons, 2002) and the International *Streptomyces* Project (ISP) as recommended by Shirling and Gottlieb (1966). A total of 49 *Streptomyces* isolates were recovered from 295 sediment samples collected from the Gulf of Aqaba, Red Sea/ Jordan. Among the four methods used to isolate *Streptomyces* (dilution, dry/ stamp, dilute/ heat, dry/ stamp+ dilute/ heat methods), method 4 (dry/stamp+ dilute/heat) yielded the highest rate of *Streptomyces* recovery (69.4%), method 3 (dilute/heat) yielded a relatively good percentage (40.8%), whereas method 1 was the least effective (20.4%). Thus method 4 was selected for the rest of experiments.

Cultural, morphological and physiological characteristics

Based on microscopic and cultural examination, the isolates, were grouped into six series based on the color of aerial mycelia; most of them belonged to the white series (60%), followed by grey and green series (16% each) (Figure 1). Most of the isolates had spiral (S) sporophore morphology (69.4%); the remaining isolates had spores in the straight or flexuous chain. Physiological data indicated that 24% of *Streptomyces* (12 isolates) produced melanin on peptone yeast extract iron agar medium, and tyrosine agar medium (Shirling and Gottlieb, 1966); only 12%(6) produced soluble pigment. Most of isolates were able to utilize D- glucose (48 out of 49 isolates), D-xylose (36), L-arabinose (29), L-rhamnose (32); D-fructose (43), D-galactose (38), D-mannitol (31) and salicin (26); whereas utilization of I-inositol (21), raffinose (5) and sucrose (18) was limited to certain isolates. For NaCl tolerance of *Streptomyces* isolates; it was found that 2% of isolates could tolerate a maximum of 4% NaCl; 24% tolerated 7% NaCl, about half of the isolates (49%) tolerated 10% NaCl, and 24% tolerated 13% NaCl.

Screening for antimicrobial activity of *Streptomyces*

Marine *Streptomyces* isolates, inoculated in starch- yeast extract- peptone broth medium, prepared with 75% seawater and incubated for 7 days, were screened for their antimicrobial activities against 10 test microorganisms using agar well diffusion method. Antimicrobial activity was determined in terms of diameter of inhibition zone surrounding the well (the size of the well was 7 mm). Inhibition zones ranged from 10 to 30 mm except for S4 isolate that gave the largest zone of inhibition against *B. subtilis* (46 mm). Results are summarized in Table 2. Among 49 *Streptomyces* isolates tested, 28 (57%) showed activity against at least one of the test microorganisms (Table 2). Among these isolates, 5 were only

Table 2. Antimicrobial activity of different color series of *Streptomyces* against test microorganisms.

Test microorganism	*Streptomyces* color series						Total number of positive isolates (percentage)
	White	Grey	Green	Blue	Red	Pink	
S. aureus	13	3	7	1	1	0	25 (89.2)
P. aeruginosa	3	1	0	1	0	0	5 (17.8)
M .luteus	9	4	4	1	0	0	18(64.0)
S. epidermidis	10	3	3	1	1	1	18(64.0)
β. hemolytic *Streptococcus*	3	1	2	0	1	0	7 (25.0)
Klebsiella	3	3	0	1	1	0	8 (28.5)
E. coli	8	2	6	1	0	1	18 (64.0)
B. subtilis	8	3	1	1	1	0	14 (50.0)
Bordetella bronchiseptica	4	1	1	0	1	0	7 (25.0)
C. albicans	4	2	0	0	1	0	7 (25.0)

Table 3. PCR detection of NRPS, PKS-I and PKS-II biosynthetic systems in the *Streptomyces* isolates.

Isolate	Active Isolates	NRPS	PKS-I	PKS-II	Inactive Isolates	NRPS	PKS-I	PKS-II
		A3F/A7R Positive	K1F/M6R positive	KSα/KSβ positive		A3F/A7R positive	K1F/M6R Positive	KSα/KSβ positive
No. of *Streptomyces* isolates	29	21	25	17	20	19	6	15

active against Gram-positive bacteria; one isolate was active against Gram- negative bacteria. Only 15 isolates showed inhibitory activity against both Gram- positive and Gram-negative bacteria, whereas 5 isolates inhibited both Gram-positive, Gram- negative and *C. albicans*. Out of the 28 isolates that exhibited antimicrobial activity, 25 isolates were active against *S. aureus*, 18 against *S. epidermidis*, 18 isolates against *Micrococcus luteus*, 17 against *E. coli ,*14 against *B. subtilis*, 8 against *Klebsiella sp*, 7 against *C. albicans*, 7 against *Bordetella bronchiseptica*, 7 against *B-hemolytic Streptococci,* and 5 against *P. aeruginosa. Streptomyces* isolate S34, showed very good activity with a wide spectrum, and thus was chosen for further studies. Furthermore, the antimicrobial activity was stable in all media (that is, starch casein nitrate broth, starch nitrate broth, and Sabouraud broth).

Detection of NRPS, PKS-I, and PKS-П genes

Amplification of NRPS, PKS-I, and PKS-II genes, using A3F/A7R, K1F/M6R and $K_{\alpha}F/K_{\beta}R$, was performed with all *Streptomyces* isolates. The prevalence of these genes is summarized in Table 3.

Identification of *Streptomyces* isolates S34

According to the description of the *Streptomyces* species recorded in Bergey's manual (2002) and International *Streptomyces* Project (Shirling and Gotlieb, 1966), isolate S34 appeared to be highly related to *S. rochei*, but requires further identification (Table 4).

Optimization of antimicrobial compounds production from *Streptomyces* S34

For the optimal production of antimicrobial activity, the following factors were optimized: Seawater content, type of medium, incubation time, pH, incubation temperature, carbon, and nitrogen sources. Results are summarized in Table 5 and Figure 2

Thermal stability and the effect of proteolytic enzymes on the antimicrobial activity of strain S34

Cell free supernatant of isolate S34 was heated to 100°C for 5, 15, 30 and 60 min. Results show that the activity of supernatant was retained during heat treatments even at 100°C for 1 h. The sensitivity of antimicrobial activity to proteolytic enzymes was tested at 37°C; the activity was stable after incubation with pepsin and trypsin for 1 h. These results suggested non proteinaceous nature of the antimicrobial compound(s) produced by isolate S34.

DISCUSSION

Several studies dealing with bioactive compounds from

Table 4 Identification of *Streptomyces* isolates S34.

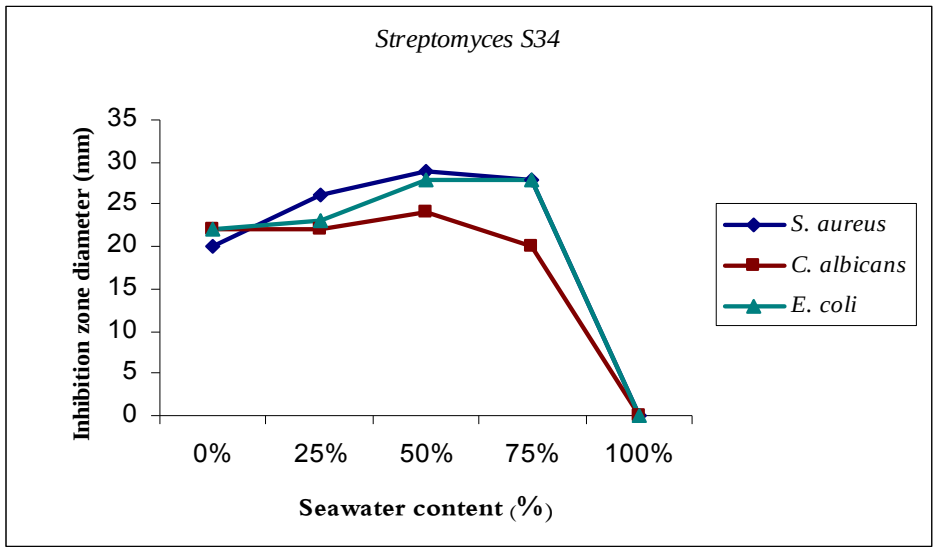
Character	*Streptomyces* S34	*Streptomyces rochei*
Gram stain	Positive	Positive
Cell shape	Filamentous	Filamentous
Color of aerial mycelium	Gray	Gray
Spore chain morphology	Spiral	Spiral
Melanoid pigment	Positive	Positive
Diffusible pigment	Negative	Negative
Growth on Czapek's medium	Good	Moderate
Carbon utilization:		
No carbon	-	-
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
D-Galactose	+	+
Raffinose	-	-
D-Mannitol	+	+
I-Inositol	+	+
Salicin	+	+
Sucrose	-	-
Antagonistic activity	Antibacterial and Antifungal	Antibacterial and Antifungal

Table 5. Optimization of antimicrobial compounds production from *Streptomyces* S34.

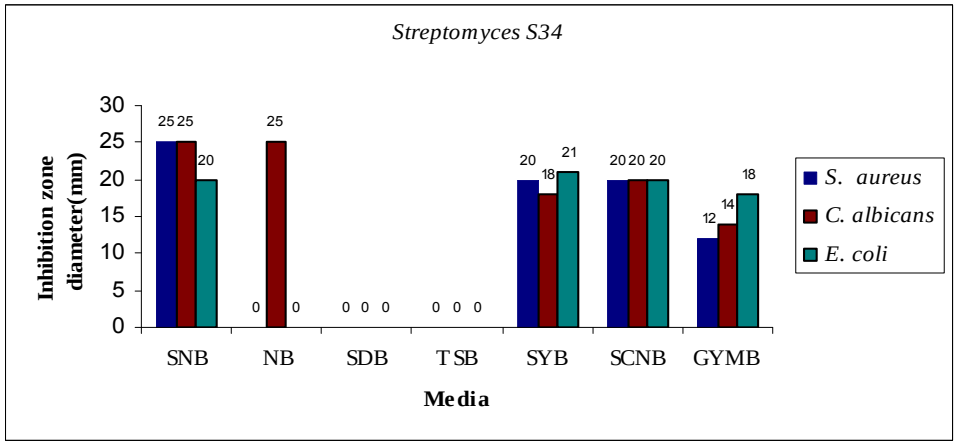
Parameter under optimization	Variation of the tested parameter	Optimum antimicrobial activity
Sea water content	0, 25, 50,75, and 100%	50%
Medium component	NB,SDB,TSB,SYB, SNB,SCNB,GYMB	SNB
Incubation period	2,3,4,5,6,8,10,12, and 14 days	4-5 days
pH	From 3.0 to 12.0 with 0.5 intervals	5.5 and 8.5-9
Temperature	From 20 to 50°C with 5 intervals	30°C
Agitation rate	From 0 to 250 with 50 differences	150-200 rpm

the genus *Streptomyces* isolated from different habitats in marine environments (sediments, invertebrates, and coral reefs) have been reported. Members of *Streptomyces*, like terrestrial counterparts, are promising source for production of bioactive compounds (Maldonado et al., 2005; Moore et al., 2005; Parthasarathi et al., 2012a, b; Haritha et al., 2012). Since the marine environment in Jordan is still unexplored and unexploited, this study was performed to isolate *Streptomyces* and investigate their antagonistic properties. *Streptomyces* isolates were identified based on cellular and colony morphology, utilization of carbon, and physiological characteristics (Holt et al., 1994). The observed properties indicated that the isolates belonged to the genus *Streptomyces*. Most of the isolates (59%) belonged to white color series, followed by grey and green color series. Dominance of white and grey color series was reported in several studies (Saadoun and Gharaibeh, 2002; Parthasarathi et al., 2012a; b).

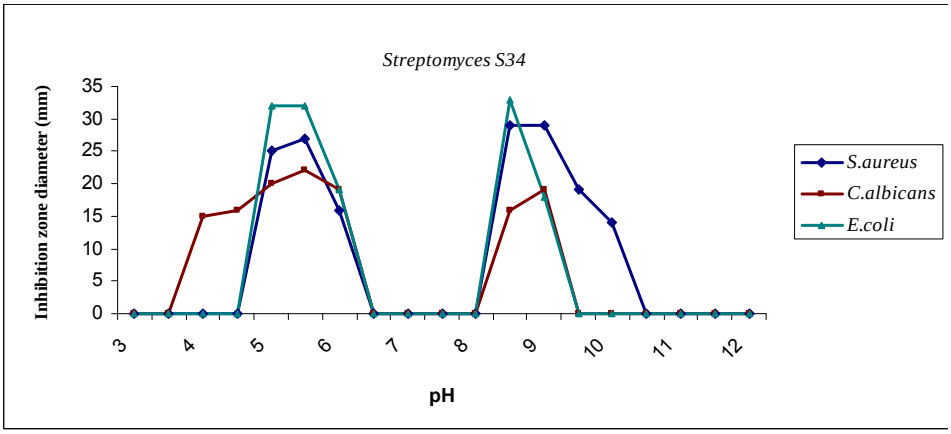
Preliminary screening of antimicrobial activity of *Streptomyces* isolates showed that more than half of our isolates (57%) were active against at least one of the test microorganisms. Similarly, the majority of *Streptomyces* isolated from soils in Jordan showed antimicrobial activity (Saadoun et al., 1999). The proportion of active isolates depends on the methods of preliminary screening and on the type of culture used (broth or agar) (Augustine et al.,



1

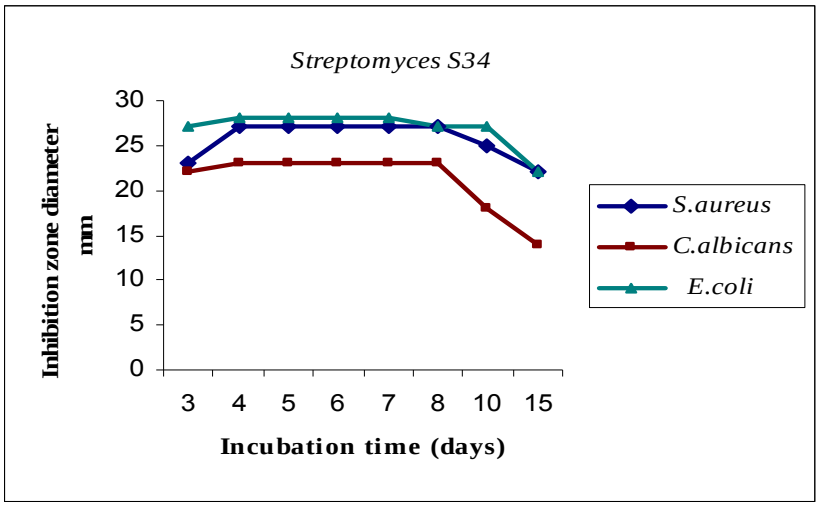


2

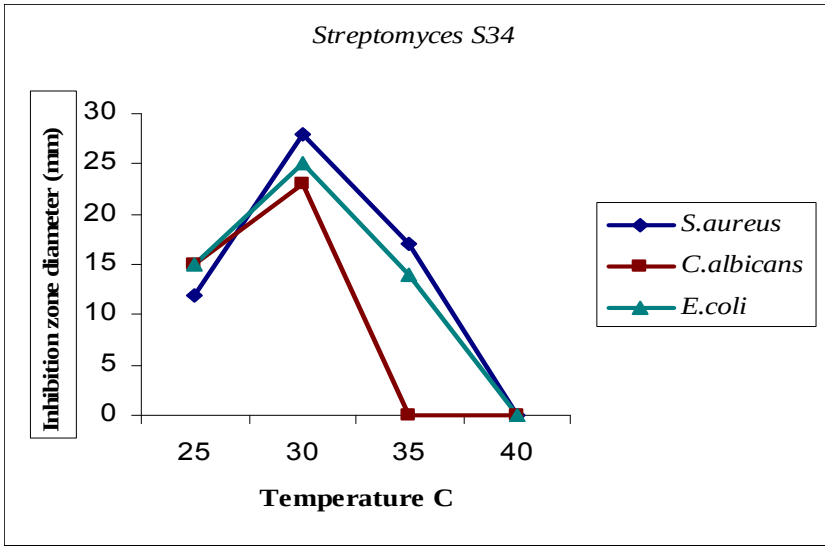


3

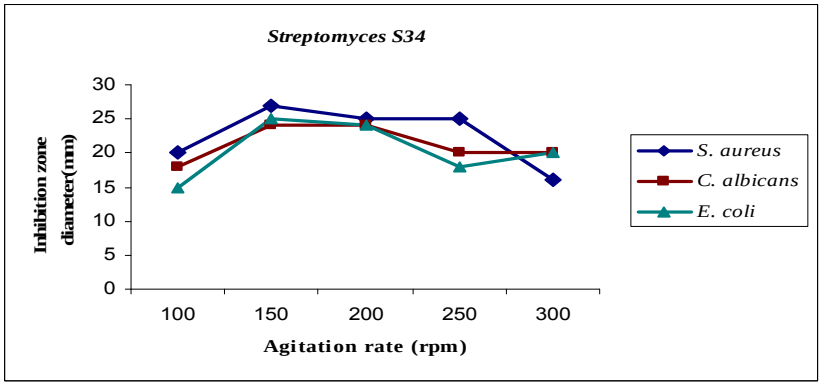
Figure 2. Optimum conditions for the production of antibacterial metabolites from *Streptomyces* S34: Sea water content (1), medium component (2), incubation period (3), pH (4), temperature (5), agitation rate (6).



4



5



6

Figure 2. Contd.

2005a, b). During screening, *Streptomyces* isolates were subjected to the same growth and incubation conditions; it appeared that each isolate required specific growth and antimicrobial production conditions (medium, temperature, pH, and agitation). In addition, the size of sample, stability of antibiotic, bioassay method and test microorganisms appear to affect the number of active isolates (Srivibool and Sukchotiratana, 2006). It was reported that *Streptomyces* isolates were more active against Gram-positive bacteria than Gram-negative bacteria (Silambarasan et al*.,* 2012; Valli et al., 2012). In this study also *Streptomyces* isolates showed a significant antimicrobial activity against *S. aureus*, *S. epidermidis*, and *B. subtilis*, than Gram-negative *P. aeruginosa*. Difference in sensitive between Gram -positive and Gram-negative bacteria might be due to the cell wall structure; the outer polysaccharide membrane present in Gram- negative bacteria which acts as lipopolysaccharide barrier; the lack of this barrier in Gram -positive bacteria makes the cell wall more susceptible (Silambarasan et al*.,* 2012; Valli et al., 2012). For this reason, the amount of antibiotic required for inhibition of Gram-positive bacteria was more than that required for Gram-negative inhibition (Selvin et al., 2004; Sahin, 2005; Srivibool and Sukchotiratana, 2006).

Screening study of the occurrence of biosynthetic pathways of metabolites is of great value to understanding the ecological impact of organisms and fitness of populations (Ehrenreich et al., 2005). Several previous studies assessed the biosynthetic potential of soil *Streptomycetes* were performed (Metsa-Ketela et al., 2002). In the present study, PCR screening of NRPS (700 bp), PKS-I (1400 bp) and PKS-II (613 bp) genes in marine *Streptomycetes* using degenerate primers revealed that NRPS genes were detected in the majority of isolates (81.6%). PKS-I and PKS-II sequences were also detected in most of the isolates tested, but with relatively lower percentage (63.2 and 65.3%, respec-tively). High prevalence of NRPS genes (68%) as well as PKS-I sequences were reported in most of the *Actinomycetes* isolated from marine sediments, of the deepest site of Mariana Trench in the western Pacific Ocean; whereas PKS-I sequences were identified in only 13% of the strains (Pathom-aree et al., 2006). Addi-tionally, NPRS and PKS genes were reported with high frequency in other marine organisms including marine and fresh water cyanobacteria (Ehrenreich et al., 2005) and from marine dinoflagellates (Snyder et al., 2005). Similarly, a study of Ayuso-Sacido and Genilloud (2005) revealed that the NRPS sequences were widely distributed in soil *Actinomycetes* (79.5%), but PKS-I was identified only in 56.7%; whereas among *Streptomyces* isolates, NPRS and PKS-I genes were detected in most of the isolates with higher frequency 97 and 79%, respectively (Ayuso-Sacido and Genilloud 2005). Also, NPRS, PKS- I and PKS-II sequences showed high occurrence in *Streptomyces* isolated from tropical soil samples (60.0, 72.4 and 69.2%, respectively) (Ayuso et al., 2005). Upon comparing the *Streptomyces* local isolates, with and without antimicrobial activity, we observed that higher detection percenttages were obtained for the PKS- I in the group of active isolates than in the group of inactive isolates (Table 4). This relationship between the occurrences of biosynthetic gene sequences and the production of antimicrobial activities was not observed for the NPRS and PKS-II sequences (Table 4). Our results differed from that obtained by Ayuso et al. (2005) who reported that the percentages of positive NRPS and PKS-I amplifications (except for PKS-II sequences) were almost two-fold higher in the active compared with the inactive group.

Ayuso-Sacido and Genilloud (2005) reported that the NPRS primers (A3F/A7R), PKS-I primers (K1F/M6R), and PKS-II primers (KSα/KSβ) amplified the highly conserved sequences of adenylation domains associated with NRPSs and ketosynthase (KS) domains associated with type I PKS. The lack of amplification of these genes in some isolates might indicate their absence or that they were less conserved, hence low homology with the primers. On the other hand, some isolates obtained in this study were negative for NPRS and PKS genes, but they showed bioactivity against test microorganisms, these results suggested that the activities detected were produced by systems other than PKS and NRPS genes, such as aminoglycoside resistance gene (Ayuso et al., 2005). Other isolates did not show any antimicrobial activity in spite of the occurrence of NPRS and PKS systems. It is possible that these detected genes may be silent (nonfunctional) (Hutchinson, 1999, 2003). Studies of sequenced genomes of *Streptomyces coelicolor* and *Streptomyces avermitilis* have demonstrated numerous silent pathways (Challis and Hopwood, 2003; Knight et al., 2003), or that the products of these genes may be involved in primary metabolism (Pathom-aree et al., 2006), or that fermentation conditions used were not optimal for antibiotic production. In fact, the genome of *Streptomyces* contained several gene clusters of NPRS and PKS genes; Pathom-aree et al., (2006) reported that the genome of *S. coelicor* contained five NPRS and three PKS-I clusters, and only four NPRS clusters have known to be involved in the synthesis of known compounds. This may indicate that a huge number of bioactive compounds are still unidentified. Of the 49 *Streptomyces* isolates, S34 showed high antimicrobial activity against test microorganisms. The isolate was identified based on the morphological and cultural characteristics. Isolate produced powdered colony on the surface of agar plate, it is Gram positive and filamentous in nature, belonged to grey color series. S34 showed similar characteristic as that of *S. rochei*.

Isolate S34 was selected to optimize the production of active metabolites. Production of antimicrobial metabolites was significantly influenced by cultural and environ-

mental factors. Influence of these factors has been evaluated in marine *Streptomyces* by several workers (Saha et al., 2005; Narayana and Vijayalakshmi, 2008; Sunga et al., 2008; Arasu et al., 2009; Singh et al., 2009; Thakur et al., 2009). In this study, isolate S34 produced heat stable non proteinaceous metabolites that have broad spectrum and high activity against pathogenic bacteria and yeast tested.

Conclusions

Marine *Streptomyces* species, isolated from the Gulf of Aqaba/Jordan, was found to be highly diverse and produced wide spectrum antimicrobial agents. The optimal medium, nutrients, pH, temperature, and other culture conditions promoted the effectiveness of the antimicrobial agents. The majority of the isolates showed activity against Gram positive bacteria, lower activity was observed toward Gram negative bacteria and yeast. *Streptomyces sp*. S34 had wide spectrum activity (it inhibited Gram-positive, Gram-negative bacteria, and yeast), strong activity, which was determined by largest inhibition zone diameter (30 mm), and antimicrobial activity at both acidic and alkaline pH (5 to 5.5 and 8 to 9.5). Furthermore, antimicrobial activity showed temperature stability. Isolate S34 produced non proteinaceus heat stable antimicrobial metabolites. It can be concluded that marine *Streptomyces* strains isolated from the Gulf of Aqaba have a great potential as a source of secondary metabolites with antibacterial activity. However, further investigation is needed to isolate and characterize the active secondary metabolites.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to the Deanship of Graduate Studies at The University of Jordan for financial support.

REFERENCES

Abussaud MJ, Alanagreh L, Abu-Elteen K (2013). Isolation, characterization and antimicrobial activity of *Streptomyces* strains from hot spring areas in the northern part of Jordan. Afr. J. Biotechnol. 12(51): 7124-7132.

Anderson AS, Wellington EM (2001). The taxonomy of *Streptomyces* and related Genera. Int. J. Syst. Evol. Microbiol. 51: 797–814.

Arasu MV, Duraipandiyan V, Agastian P, Ignacimuthu S (2009). In vitro antimicrobial activity of *Streptomyces spp. ERI-3* isolated from Western Ghats rock soil (India). J. Mycol. Med. 19: 22-28.

Augustine SK, Bhavsar SP, Kapadnis BP (2005a). A non-polyene antifungal antibiotic from *Streptomyces albidoflavus PU 23*. J. Biosci. 30(2): 201–211.

Augustine SK, Bhavsar SP, Kapadnis BP (2005b). Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei AK 39*. Indian J. Med. Res. 121: 164-170.

Ayari A, Morakchi H, Djamila KJ (2012). Identification and antifungal activity of *Streptomyces sp.* S72 isolated from Lake Oubeira sediments in North-East of Algeria. Afr. J. Biotechnol. 11(2): 305-311.

Ayuso A, Clark D, González I, Salazar O, Anderson A, Genilloud O (2005). A novel *actinomycete* strain de-replication approach based on the diversity of polyketide synthase and nonribosomal peptide synthetase biosynthetic pathways. Appl. Microbiol. Biotechnol. 67: 795-806.

Ayuso-Sacido A, Genilloud O (2005). New PCR primers for the screening of NRPS and PKS-I systems in *actinomycetes*: detection and distribution of these biosynthetic gene sequences in major taxonomic groups. Microb. Ecol. 49: 10–24.

Benedict RG, Pridham TG, Lindenfelser LA, Hall HH, Jackson RW (1955). Further studies in the evaluation of carbohydrate utilization tests as aids in the differentiation of species of *Streptomyces*. Appl. Microbiol. 3(1): 1–6.

Buchanan R, Gibbons NE (2002). Bergeys Manual of determinative bacteriology. 10th Ed. Williams and Wilkins Co. Baltimore.

Bull AT, Stach JEM, Ward AC, Goodfellow M (2005). Marine *actinobacteria* : Perspectives, challenges, future directions. Antonie van Leeuwenhoek 87: 65-79.

Challis GL, Hopwood DA (2003). Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. Proc. Natl. Acad. Sci. USA. 25: 14555-14561.

Dharmaraj S (2010). Marine *Streptomyces* as a novel source of bioactive substances. World J. Microbiol. Biotechnol. 26: 2123–2139.

Ehrenreich IM, Waterbury JB, Webb EA (2005). Distribution and diversity of natural product genes in marine and freshwater *cyanobacterial* cultures and genomes. Appl. Environ. Microbiol. 71(11): 7401–7413.

Haritha R, SivaKumar K, Swathi A, Jagan Mohan YSYV, Ramana T (2012). Characterization of marine *Streptomyces carpaticus* and optimization of conditions for production of extracellular protease. Microbiol. J. 2 (1): 23-35.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's manual of determinative bacteriology. 9th Ed. Baltimore Williams and Wikins. pp. 518-537.

Hopwood DA (1997). Genetic contributions to understanding polyketide synthases. Chem. Rev. 97: 2465-2497.

Hutchinson CR (1999). Microbial polyketide synthases: More and more prolific. Proc. Natl. Acad. Sci. 96: 3336-3338.

Hutchinson CR (2003). Polyketide and non-ribosomal peptide synthases: Falling together by coming apart. PNAS100 (6): 3010–3012.

Jayaprakashvel M (2012). Therapeutically active biomolecules from marine *Actinomycetes*. J. Mod. Biotechnol. 1(1): 1–7.

Jensen PR, Gontang E, Mafnas C, Mincer TJ, Fenical W (2005a). Culturable marine *actinomycete* diversity from tropical Pacifi c ocean sediments. Enviromen. Microbiol. 7: 1039–1048.

Jensen PR, Mincer TJ, Williams PG, Fenical W (2005b). Marine *actinomycete* diversity and natural product discovery. Antonie van Leeuwenhoek 87: 43-48.

Knight V, Sanglier JJ, DiTullio D, Braccili S, Bonner P, Waters J, Hughes D, Zhang L (2003). Diversifying microbial natural products for drug discovery. Appl. Microbiol. Biotechnol. 62: 446–458.

Labeda DP, Goodfellow M, Brown R, Ward AC, Lanoot B, Vanncanneyt M, Swings J, Kim SB, Liu Z, Chun J, Tamura T, Oguchi A, Kikuchi T, Kikuchi H, Nishii T, Tsuji K, Yamaguchi Y, Tase A, Takahashi M, Sakane T, Suzuki KI, Hatano K (2012). Phylogenetic study of the species within the family *Streptomycetaceae*. Antonie van Leeuwenhoek 10: 73–104.

Laidi R, Kansoh A, Elshafei A, Sheikh B. (2006). Taxonomy, identification, and biological activity of novel isolate of *Streptomyces lendae*. Arab J. Biotochnol. 9(3): 427-436

Lam KS (2006). Discovery of novel metabolites from marine *actinomycetes*. Curr. Opin. Microbiol. 9: 245–251.

Maldonado LA , Stach JEM, Pathom-aree W, Ward AC, Bull AT, Goodfellow M (2005). Diversity of cultivable *actinobacteria* in geographically widespread marine sediments. Antonie van Leeuwenhoek 87: 11–18.

Metsa-Ketela M, Halo L, Munukka E, Hakala J, Mantsala P, Ylihonko K (2002). Molecular evolution of aromatic polyketides and comparative sequence analysis of polyketide ketosynthase and 16S ribosomal DNA genes from various *Streptomyces* species. Appl. Environ. Microbiol. 68(9): 4472–4479.

Metsa-Ketela M, Salo V, Halo L, Hautala A, Hakala J, Mantsala P, Ylihonko K (1999). An efficient approach for screening minimal PKS genes from *Streptomyces*. FEMS Microbiol. Lett. 180(1): 1-6.

Mincer TJ, Jensen PR, Kauffmann CA, Fenical W (2002). Widespread and persistent populations of a major new marine *actinomycete* taxon in ocean sediments. Appl. Environ. Microbiol. 68: 5005–5011.

Moore BS, Kalaizis JA, Xiang L (2005). Exploiting marine *actinomycete* biosynthetic pathways for drug discovery. Antonie van Leeuwenhoek 87: 49-57.

Narayana KJP, Vijayalakshmi M (2008). Optimization of antimicrobial metabolites production by *Streptomyces albidoflavus*. Res. J. Pharmacol. 2(1): 4-7.

Newman DJ, Cragg MG (2007). Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70: 461-477.

Olano C, Mendez C, Salas JA (2009). Antitumor compounds from marine *actinomycetes*. Mar. Drugs 7: 210-248.

Parthasarathi S, Sathya S, Bupesh G, Durai Samy R, Ram Mohan M, Selva Kumar G, Manikandan M, Kim CJ, Balakrishnan K (2012b). Isolation and characterization of antimicrobial compound from marine *Streptomyces hygroscopicus* BDUS 49. World J. Fish Mar. Sci. 4(3): 268-277.

Parthasarathi S, Sathya S, Bupesh G, Manikandan M, Kim CJ, ManikandanT, Balakrishnan K (2012a). Isolation, characterization and extraction of antimicrobial compounds from marine *Actinomycete Streptomyces hygroscopicus* BDUS 49. Res. J. Biotechnol. 8(3): 40-49.

Pathom-aree W, Stach JEM, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006). Diversity of *actinomycetes* isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. Extremophiles 10: 181–189.

Pridham TG, Gottlieb D (1948). The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. J. Bacteriol. 56: 107-114.

Saadoun I, Al-Momani F, Malkawi HI, Mohammad MJ (1999). Isolation, identification and analysis of antibacterial activity of soil *Streptomycetes* isolates from north Jordan. Microbios 100: 41-46.

Saadoun I, Gharaibeh R (2002). The *Streptomyces* flora of Jordan and its' potential as a source of antibiotics active against antibiotic-resistant Gram-negative bacteria. World J. Microbiol. Biotechnol. 18: 465-470.

Saadoun I, Rawashdeh R, Dayeh T, Ababneh Q, Mahasneh A (2007). Isolation, characterization and screening for fiber hydrolytic enzymes-producing Streptomycetes of Jordanian forest soils. Biotechnology 6(1): 120-128.

Saadoun I, Wahiby L, Ababneh Q, Jaradat Z, Massadeh M, Al-Momani F (2008). Recovery of soil streptomycetes from arid habitats in Jordan and their potential to inhibit multi-drug resistant *Pseudomonas aeruginosa* pathogens. World J. Microbiol. Biotechnol. 24(2): 157-162.

Saha M, Ghosh DJr, Ghosh D, Garai D, Jaisankar P, Sarkar KK, Dutta PK, Das S, Jha T, Mukherjee J (2005). Studies on the production and purification of an antimicrobial compound and taxonomy of the producer isolated from the marine environment of the Sundarbans. Appl. Microbiol. Biotechnol. 66(5): 497-505.

Sahin N (2005). Antimicrobial activity of *Streptomyces* species against mushroom blotch disease pathogen. J. Basic Microbiol. 45(1): 64–71.

Savic M, Vasiljevic B (2006). Targeting polyketide synthase gene pool within *actinomycetes*: new degenerate primers. J. Ind. Microbiol. Biotechnol. 33(6): 423-430.

Selvin J, Joseph S, Asha KRT, Manjusha WA, Sangeetha VS, JayaseemaDM, Antony MC, Denslin Vinitha AJ (2004). Antibacterial potential of antagonistic *Streptomyces* sp. isolated from marine sponge *Dendrilla nigra*. FEMS Microbiol. Ecol. 50 (2): 117-122.

Shirling EB, Gottlieb D (1966). Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313 – 340.

Silambarasan S, Praveen Kumar E, Murugan T, Saravanan D, Balagurunathan R (2012). Antibacterial and antifungal activities of *Actinobacteria* isolated from Rathnagiri hills. J. Appl. Pharm. Sci. 2(10): 99-103.

Singh LS, Mazumder S, Bora TC (2009). Optimization of process parameters for growth and bioactive metabolite produced by a salt-tolerant and alkaliphilic actinomycete, *Streptomyces tanashiensis* strain A2D. J. Mycol. Med. 19: 225-233.

Sirisha B, Haritha R, Jagan Mohan YSYV, Siva Kumar K, Ramana T (2013). Bioactive compounds from marine *actinomycetes* isolated from the sediments of bay of Bengal. Int. J. Pharm. Chem. Biol. Sci. 3(2): 257-264.

Snyder RV, Guerrero MA, Sinigalliano CD, Winshell J, Perez R, Lopez JV, Rein KS (2005). Localization of polyketide synthase encoding genes to the toxic dinoflagellate *Karenia brevis*. Phytochemistry 66: 1767–1780.

Srivibool R, Sukchotiratana M (2006). Bioperspective of *actinomycetes* isolates from coastal soils: A new source of antimicrobial producers. J. Sci. Technol. 28(3): 493-499.

Sunga MJ, Teisan S, Tsueng G, Macherla VR, Lam KS (2008). Seawater requirement for the production of lipoxazolidinones by marine *actinomycete* strain NPS8920. J. Ind. Microbiol. Biotechnol. 35: 761–765.

Thakur D, Bora TC, Bordoloi GN, Mazumdar S (2009). Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by *Streptomyces sp*. 201. J. Med. Mycol. 19: 161-167.

Valli S, Svathi Sugasini S, Aysha OS, Nirmala P, Vinoth Kumar P, Reena A (2012). Antimicrobial potential of *Actinomycetes* species isolated from marine environment. Asian Pac. J. Trop. Biomed. 2(6): 469-473.

Wu SJ, Fotso S, Li F, Qin S, Kelter G, Fiebig H, Laatsch H (2006). *N*-Carboxamido-staurosporine and Selina-4(14), 7(11)-diene- 8,9-diol, New Metabolites from a Marine *Streptomyces* sp. J. Antibiot. 59(6): 331–337.