The Asia Journal of Applied Microbiology
2018 Vol. 5, No. 1, pp. 1-14
ISSN(e): 2313-8157
ISSN(p): 2409-2177
DOI: 10.18488/journal.33.2018.51.1.14

ANTIBACTERIAL ACTIVITY OF *STREPTOMYCES TUNISIENSIS* MG520500 ISOLATED FROM SOIL SAMPLES

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Article History
Received: 29 December 2017
Revised: 22 January 2018
Accepted: 26 January 2018
Published: 29 January 2018

Keywords
Antibacterial
MDRB
Streptomyces tunisiensis
Cytotoxicity
Gram positive
Gram negative.

ABSTRACT

In the present study seven agriculture soil samples were collected from Dakahlia Governorate, Egypt. Twenty five actinomycete isolates were purified and selected according to their culture characteristics on starch nitrate agar medium. These purified isolates were examined for antibacterial activity against multidrug resistant bacteria (MDRB). Gram positive (E. faecalis and MRSA) and Gram negative E. coli. Among all tested actinomycete isolates only SA-13 isolate could inhibit the growth of all tested bacteria. All morphological, physiological and biochemical results of SA-13 isolate indicated that it belongs to the genus Streptomyces. The comparative analysis of 16S rDNA gene sequence and phylogenetic relationship showed that SA-13 isolate lies with strain *Streptomyces tunisiensis* CN-207. SA-13 submitted to the GenBank NCBI with the accession number, MG520500. Different optimization parameters were studied to enhance the antibacterial activity. The optimum conditions were temperature 30°C, pH range7-8, inoculum size 6% v/v on modified riched medium and shaking at 200rpm. Cytotoxic activity of crude extract showed IC50 200µg/ml. Our results proves that; this isolate exhibit antibacterial activity against MDRB. Additional studies of purification and identification of bioactive compound may be useful for discovering a novel antibacterial compound.

Contribution/Originality: This study documents *Streptomyces tunisiensis* SA-13 is powerful soil actinomycetes having a broad spectrum activity against resistant strains of pathogenic bacteria (ESKAPE).

1. INTRODUCTION

Soil microorganisms provide an important source for discovery of therapeutically important active compounds. Among them, actinomycetes are an important group [1]. The order actinomycetales is comprised of approximately 80 genera, nearly all from soils origin, where they present mainly as saprophytes, water and associated plants showing marked chemical and morphological diversity, but from a distinct evolutionary line [2]. A large number of actinomycetes have been screened from soil over years, accounting for about 75% of available secondary metabolites [3]. Actinomycetes are promising source of many bioactive metabolites [4-7] which have many important applications. It has been assessed that about one-third of the thousands of natural antibiotics have been isolated from actinomycetes [8].

The continuous release of new generations of multi drug resistant pathogenic strains increases the demand to discover new antibiotics effective against resistant pathogenic strains. So, we need to screen more and more of soil

actinomycetes from different habitats for antimicrobial activity in the potential of obtaining new antibiotics active against multi drug resistant Strains.

2. MATERIAL AND METHODS

2.1. Soil Sampling and Pretreatment

Soil samples were collected from different area of Dakahlia Governorate (geographical coordinates: latitude 30°51'18.17"N and longitude 31°16'53.00"E), Cairo, Egypt. Samples were collected by inserting a sterilized spatula into the sediments from different locations including field, near plant surface, mining area, farm, and well. The samples were collected at depth 6-12 inch of the ground surface and placed in sterile bags and transported immediately to the laboratory. These soil samples were air-dried, crushed, and sieved prior to use for isolation [9].

2.2. Microorganisms

Multi drug resistant (MDR) bacteria were *Staphylococcus aureus* MRSA, *Enterococcus feacalis* and *Escherichia coli* in addition to standard strains *Staphylococcus aureus*-ATCC-*29213* and *Escherichia coli*-ATCC-*6539*. These bacteria were kindly supplied by the staff member of bacteriology lab at microbiology department, faculty of science, Al_Azhar University.

Antibiotic susceptibility profile of MDRB was repeated for confirmation of resistance using agar diffusion method, according to clinical and laboratory standard institute (CLSI) guideline [10].

2.3. Isolation and Purification of Actinomycetes

Actinomycetes were isolated by serial dilution method according to Janaki, et al. [11] with some modifications. Stock solution of prepared soil samples were performed using serial dilution method and inoculated on starch nitrate agar plates. The inoculated plates were incubated at 28°C for 2 to 3 weeks. The appeared colonies were picked and purified according to color, dryness, rough, convex colony [12].

2.4. Antibacterial Activity Evaluation of Purified Actinomycete Isolates Using Agar Plug Assay

Three discs (6mm diameter) of purified actinomycetes culture grown on starch nitrate agar at 28°C for 14 day were placed on the surface of seeded Mueller–Hinton agar plates (Hi-Media - Mumbai) with MDRB and standard strains (0.5 McFarland standards) [13]. The prepared plates were incubated at 37°C for 24 h and the inhibition zone diameter was measured(mm).

2.5. Antibacterial Screening Using Broth Culture of Selected Actinomycetes

Based on the results obtained from agar plug assay, the isolates that exhibit antibacterial activity were subjected to secondary screening for evaluation of their activity using starch nitrate broth [14]. The selected actinomycetes culture were inoculated into 250 ml Erlenmeyer flask containing 50 ml of broth culture and placed into rotary shaker incubator at 150 rpm and 28°C. After incubation (48h), the seed culture (4% v/v) was transferred to 1000 ml Erlenmeyer containing 200ml broth culture and incubated under the same condition for 14 days. After incubation, 100µl of cell free filtrate was assessed for activity using agar well method. Three replicates were made and the mean diameters of inhibition zone (mm) was calculated.

2.6. Characterization of the Most Potent Actinomycetes Isolate

The most active actinomycete isolate was characterized according to morphological, physiological and biochemical characteristics [15]. Microscopic examination was performed using light microscope (Optika, Italy) through cover slip technique [16] and scanning electron microscopy (SEM) (JEOL Technics Ltd, Japan) at the regional center for mycology and biotechnology, Al-Azhar University, Cairo [17] Culture characteristics were

screened on different International Streptomyces Project (ISP) media: tryptone yeast glucose agar medium (ISP-1), inorganic salts starch agar (ISP-4), Peptone yeast extract agar (ISP-6), malt extract agar (ISP-2), glycerol asparagine agar (ISP-5), oat meal agar (ISP-3) and Tyrosine agar (ISP-7) after incubation at 28°C for 10 days. Actinomycete isolate was evaluated for their ability to utilize different carbon and nitrogen sources in ISP-9 media. Salt tolerance was examined on starch nitrate medium at different concentrations of NaCl; 0, 1, 2, 3, ... 10% (w/v). pH and temperature ranges were studied on starch nitrate medium [18]. Tolerance to toxic materials was performed including Sodium azide 0.01 & 0.02% (w/v), Phenol 0.1%(w/v), Crystal violet 0.001%(w/v) and growth on czapek's medium according to Atta, et al. [19]. Biochemical parameters including catalase, citrase, oxidase, lipase, caseinase, gelatinase, lecithinase, urease production, cellulose decomposition of tyrosine and pectin, H_2S production were performed [20]. The identification scheme of actinomycete isolate was followed according to Bergey's Manual of Determinative Bacteriology [21]. Molecular identification was done using 16S rDNA analysi [19].

2.7. Fermentation Studies

2.7.1. Effect of Different Media on the Production of Antibacterial Metabolite(s)

Ten different culture media were used. These media were starch nitrate medium [12] Bennett's medium [22] starch casein medium [15] ISP-2 medium, ISP-5 medium [23] ISP-4 medium, modified marine medium [24] riched medium, Czapek's medium and potato dextrose medium. After incubation at 28°C, 150rpm for 15 days, the clear filtrate of All flasks were assessed for antibacterial activity to select the best medium for production. The antibacterial activity was tested by agar well diffusion method against MDRB.

2.8. Effect of Culture Method, Different pH, Temperature and Inoculum Size on the Production of Antibacterial Metabolite

To study the effect of culture methods on the productivity of SA-13 isolate, 6 conical flasks containing the best production medium were inoculated with 4% (v/v) seed culture and divided into 2 groups (3 flasks each) the first group was incubated under static condition at 28℃ while the second group was incubated under shaking condition 150rpm for 8 days [25].

The effect of pH value on the production of antibacterial metabolites by SA-13 isolate was studied at different pH values (5–9) and incubated on rotary shaker at 28℃ for 8 days. The optimum pH of culture medium was selected for the next parameter of optimization. The optimum temperature was studied at different temperatures 15, 20, 25, 30, 35, 40 and 45℃ [26].

To evaluate the effect of inoculum size on the production of bioactive metabolites, sterile production media medium was inoculated with 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% (v/v) of seed culture while maintaining all optimized parameters fixed [27].

Antibacterial activity of all parameters was evaluated as mentioned above. All experiments were done in triplicate. The mean and standard error values were calculated.

2.9. Cytotoxicity Assay

Cell Line: Vero cell line (ATCC CCL-81) used during this study was obtained from cell culture Laboratory at the Egyptian Company for Vaccine Sera and Drug (EGYVAC), Dokky, Agouza, Giza, Egypt.

Vero cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) - Sigma-Aldrich supplemented with 10% Fetal Bovine Serum (FBS). The cells were incubated at 37°C in a humidified 5% CO2.

Crude Extract: active crude extract was obtained from broth culture of SA-13 isolate by mixing the clear filtrate of isolate with di-ethyl ether in ratio 1:1 and incubated on shaker 150 rpm for 2 hours. The solvent layer was

obtained and evaporated using rotary evaporator to obtain concentrated crude extract. Antibacterial activity for crude extract was tested before evaluating cytotoxicity.

2.10. MTT Assay

The crude extract of actinomycete isolate SA-13 was tested for *in vitro* cytotoxicity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [28]. Briefly, different concentration of crude extract was performed in DMEM using Double fold dilution method. The prepared concentrations of crude extract were filtered through syringe filter 0.22µL for sterilization.

The cultured Vero cells were harvested by trypsinization in 50mL falcon tube. Then, the cells were seeded at a density of 1×10^6 cells/mL. 100µL of cell suspension was pipetted into each well of 96-well plates from row B to row H. Each concentration was replicated 3 times and incubated at 37°C in a humidified 5% CO_2 for 24 h. After the incubation period, MTT (20µL of 5mg/mL) was added into each well and the cells were incubated for another 2-4 h until formation of formazan crystals. The medium together with MTT (190µL) were decanted from the wells. Then, 100µL of Di-Methyl Sulfoxide (DMSO) was added to dissolve formazan crystals. The absorbance was measured at 540nm in a microtiter plate reader and the percentage cell viability (CV) was calculated using the formula:

$$CV = \frac{\text{Average abs. of duplicate fraction well}}{\text{Average abs of control well}} \text{ X 100 \%}$$

A dose-response curve was plotted to enable the calculation of the concentrations that kill 50% of the Vero cells (IC50)

2.10. Statistical Analysis

The data obtained were determined to be normally distributed. Homogeneity of variances was assessed using Levene's test. Statistical analysis was also performed using a one-way classification of ANOVA where differences were regarded as statistically significant with probability $p<0.05$ using Minitab 17 software extended with a statistical package and Microsoft™ Excel® 2013 to analyze the data.

3. RESULTS AND DISCUSSION

3.1. Antibiotic Sensitivity Confirmation of MDRB

Antibiotic sensitivity profile of MDRB was performed in order to confirm their resistance to different antibiotics. The antibiotic sensitivity profile was performed using 19 antibiotics represent different classes of antibiotics that are commercially available in form of paper disks (Table 1).

3.2. Isolation, Purification and Antibacterial Screening of Actinomycetes Cultures against MDRB

The present work involves the isolation of actinomycetes from seven agriculture soil samples obtained from different area of Dakahlia Governorate (geographical coordinates: latitude 30°51'18.17"N and longitude 31°16'53.00"E), Cairo, Egypt. A total of 25 actinomycetes were isolated and purified based on their morphology (powdery and leathery consistency and stick firmly to agar surface) and capability to grow on starch nitrate agar medium.

Due to the risk of multidrug resistant pathogens emergence, there are an urgent need for discovery of new antibiotics active against MDRB [12]. So, the screening and isolation of promising actinomycetes with potential antibiotics is still an important area of research [29].

Table-1. Antibiotic sensitivity profile of MDRB

Antibiotic \ Bacteria	Abbr.	Enterococcus faecalis		Escherichia coli		Staphylococcus aureus	
Penicillin 10 µg/ml	P	0	R	0	R	0	R
Rifampin 5µg/ml	RA	0	R	0	R	11	R
Amikacin30 µg/ml	AK	0	R	17	S	10	R
Kanamycin 30 µg/ml	K	0	R	0	R	0	R
Rifampicin 30 µg/ml	RF	0	R	0	R	22	I
Erythromycin 15 µg/ml	E	0	R	0	R	0	R
Bacitracin 10 µg/ml	B	0	R	0	R	0	R
Chloramphenicol 30 µg/ml	C	0	R	0	R	0	R
Gentamycin 10 µg/ml	CN	16	S	0	R	17	□
Amoxicillin/Clavulanic acid 30 µg/ml	AMC	0	R	0	R	0	R
Ciprofloxacin 5 µg/ml	CIP	0	R	0	R	0	R
Trimethoprime/Sulfomethoxazole 25 µg/ml	SXT	0	R	15	I	0	R
Clindamycin 2 µg/ml	DA	0	R	0	R	0	R
Nalidixic acid 30 µg/ml	NA	22	S	0	R	0	R
Vancomycin 30 µg/ml	VA	□	R	0	R	0	R
Tetracycline 30 µg/ml	TE	0	R	0	R	11	R
Neomycin 30 µg/ml	N	15	I	14	R	0	R
Methicillin 5 µg/ml	MET	0	R	0	R	0	R
Cefuroxime30 µg/ml	CXM	22	I	10	R	0	R

"R" indicate resistant, "I": intermediate, "S": sensitive

The first screening of antibacterial activity against both MDRB and standard strains showed only 9 actinomycete isolates exhibited antagonistic property against at least one of the tested bacteria. Actinomycete isolates coded SA-14, SA-16 and SA-13 were found to be most active tested bacteria. So, these isolates were selected for second screening. Agar well diffusion results of the filtrate for the selected three actinomycetes showed that SA-13 isolate exhibited a considerable activity against all multidrug resistant bacteria and standard strains. The antibacterial activities of filtrate for both of SA-14 and SA-16 isolates were active against most of tested bacteria except *Enterococcus feacalis* (Table 2). Depend on these results, the isolate SA-13 was selected as the most potent bioactive isolate.

Table-2. Antibacterial activity of selected Actinomycete isolates.

No.	Bacteria	Mean of inhibition zone diameter (mm)		
		SA-14	SA-16	SA-13
1	MRSA	10	12	15
2	*E. faecalis*	0	0	15
3	*Staphylococcus aureus-ATCC-29213*	11	17	16
4	*E. coli*	15	14	14
5	*E. coli-ATCC-25922*	14	15	18

The most frequent multidrug resistant (MDR) bacteria were *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Enterobacter* sp [29, 30] The discovery of new active compounds from normal soil-derived actinomycetes is declining because of the repeating in the isolation of known actinomycetes and antibiotics [27]. During this study we tried to get a novel antibacterial metabolite(s) from the screened actinomycete isolates against MDRB. Due to the resistance of indicator bacterial strains, out of the 25 actinomycete isolates subjected to the preliminary and secondary antibacterial screening, only three isolates (12 %) were exhibited a broad-spectrum activity against most of tested bacterial strains.

3.3. Identification of the Actinomycete SA-13 Isolate

3.3.1. Morphological Characteristics

Microscopic examination of the most potent actinomycete isolate SA-13 revealed that; the isolate exhibited typical characteristics of the genus *Streptomyces* [16]. Typically, the colonies were observed to be convex and some part of the aerial mycelia and spore chains were distinguishable around the colony edge. Substrate mycelium was well-developed without fragmentation. From scanning electron microscopy it can be deduces that the strain undergoes various stages of morphological development (Fig. 1). After 14 days growth, *Rectiflexible*-type spore-chain morphology with smooth spores appeared. This isolate has distinguishable sporangia with Smooth and almond-shaped spores.

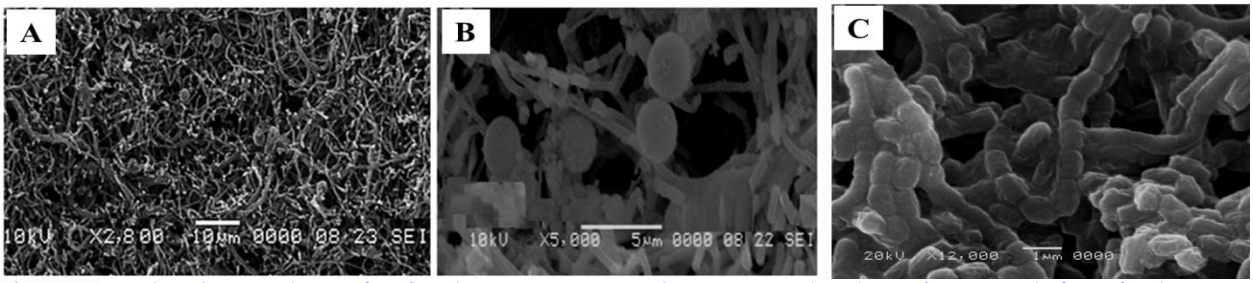

Figure-1. Scanning electron micrographs of strain SA-13 on yeast-malt extract agar (ISP2) at 28°C. Sporangiophores bearing mature sporangia are visible in (**A**,**B**). Straight spore chains with smooth surface (**C**).

3.4. Culture Characteristics

Culture characteristics of SA-13 isolate were observed on different ISP media. This isolate was grown well on ISP1, ISP3, ISP4 and ISP7 media and moderate growth was observed on ISP2, ISP5 and ISP6. It was produced white powdery colonies on the surface of agar while reversed side was yellow to pale brown (Table 3) and (Fig.2).

Table-3. Culture characteristics of the actinomycete isolate SA–13 grown on different ISP-media

Types of media	Growth	Color of substrate mycelium	Color of aerial mycelium	Color of diffusible pigments
tryptone yeast extract (ISP – 1)	Weak	Grayish yellow	White	None
Yeast extract (ISP– 2)	Good	Pale Greyish	Grey	Pinkish gray
Oat meal agar (ISP – 3)	Good	Light Gray	Light Gray	Slightly purple
Inorganic salts-starch agar (ISP – 4)	Good	Slightly purple	Light Gray	Slightly purple
Glycerol asparagine agar (ISP – 5)	Good	Light Gray	White	Slightly purple
Peptone yeast extract iron agar (ISP6)	No	-	-	-
Tyrosine agar (ISP – 7)	Moderate	Dark yellow	Light Gray	yellow brown

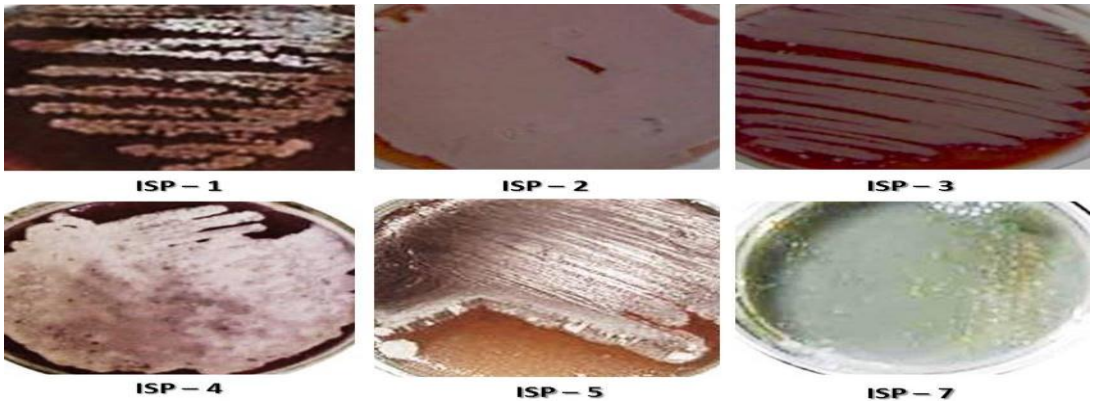

Figure-2. Culture characteristics of the actinomycete isolate SA–13 grown on different ISP-media Physiological and biochemical characteristics

The isolate was grown on a wide range of sugars and amino acids added to ISP-9 medium as a sole carbon and nitrogen sources respectively. Growth of isolate SA-13 was observed at a range of pH (6-8) but the best growth was exhibited at pH (7-8). Also, the temperature range was (20℃ – 40℃). NaCl concentrations suppressed the growth starting from 8% and above. The growth was inhibited by each of sodium azide, phenol and crystal violet. The isolate was succeeded to grow on czapek's medium as shown in Table 4.

Table-4. Physiological characteristics of actinomycete isolate SA-13

Characteristics	Results	Characteristics	Results
Utilization of carbon sourc□s		Growth at different temperatures (˚C)	
D- Glucose	++	15	-
L-Rhamnose	-	20	+
D-Xylose	±	25	++
Mannitol	++	30	++
Inositol	-	35	+
S□crose	+	40	+
Arabinose	+	45	-
Cellulose	-	Growth at different concentration of NaCl (%)	
Fructose	+	1%	++
Starch	++	□%	++
Utili□ation of amino acids		3%	++
L-Asparagine	+	4%	++
L-Cysteine	-	5%	++
L-Valine	-	6%	++
L-Therionine	+	7%	+
L-Phenylalanine	+	8%	-
L-Methionine	+	9%	-
L-Histidine	-	10%	-
L-Arginine	+		
Growth at different pH values		Tolerance to toxic substances	
5	-	Sodium azide 0.01 %(w/v)	+
6	+	Sodium azide 0.02 %(w/v)	-
7	++	Phenol 0.1%(w/v)	-
8	++	Crystal violet 0.001%(w/v)	-
9	-	Growth on czapek's medium	+

3.5. Biochemical Characteristics

Biochemical characteristics explain the behavior of the isolate SA-13 toward different substrates where, it could hydrolyze starch, casein but not lipid, gelatin, lecithin and urea. Degradation of esculin was positive whereas tyrosine degradation was negative table (5).

Table-5. Biochemical characteristics of actinomycete isolate SA-13.

No.	Test	Results
1	Lipid hydrolysis	-
2	Starch hydrolysis	+
3	Gelatin hydrolysis	-
4	Casein hydrolysis	+
5	Tyrosine degradation	-
6	Urea degradation	-
7	Pectin degradation	-
8	Esculin degradation	+
9	Citrate utilization	+
10	Motility test	-
11	H_2S production	-
12	Lecithin degradation	-
13	Catalase	-
14	Oxidase	+

3.6. Molecular Identification

The partial 16S rDNA sequence (700 nucleotides) of SA-13 isolate was determined and deposited in GenBank under the accession number MG520500. This sequence was aligned with those of *Streptomyces* reference species available in the GenBank database, which confirmed the identification of SA-13 isolate at the genus level. The similarity level was 90% with *Streptomyces tunisiensis* strain CN-207 as shown in Fig. 4.

Based on 16S rDNA gene sequence analysis, DNA–DNA relatedness studies and phenotypic features, strain CN-207T was delineated from its phylogenetically related *Streptomyces* species and so should be considered to represent species of the genus *Streptomyces tunisiensis*.

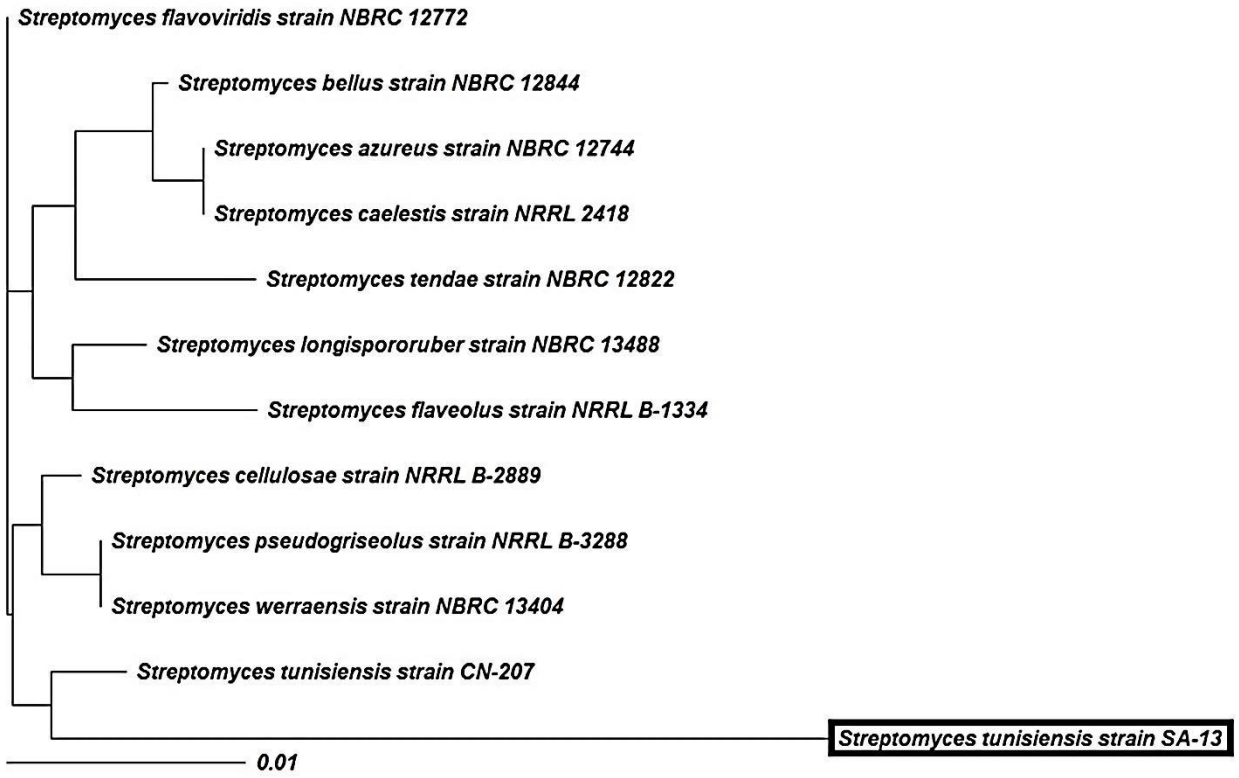

Fig-3. Phylogenetic tree showing the relations between SA-13 isolate and the most closely related type strains of Streptomyces based on 16S rDNA sequence alignment.

The present findings support the view that soil is a valuable resource for the isolation of novel Streptomyces taxa. Strain SA-13 produced antibacterial metabolites with promising features for the development of new antibiotics active against MDRB.

3.7. Fermentation Studies

3.7.1. Effect of Cultivation Methods

Effect of different cultivation methods on the production of bioactive metabolite(s) by *Streptomyces tunisiensis* SA-13 was studied throughout their cultivation in both static and shaking (submerged) conditions. It was found that the submerged cultivation was shown significant increasing in the antibacterial activity (inhibition zone ranged from 14-16 mm) than that of static condition (inhibition zone ranged from 14 – 15 mm) (Fig. 4).

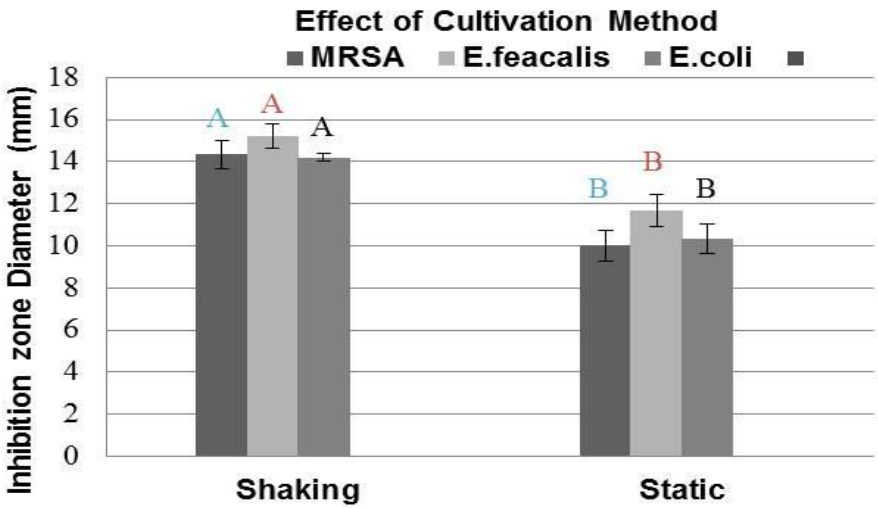

Figure-4. Effect of cultivation methods on the antimicrobial activity of *Streptomyces tunisiensis* SA-13

Culturing condition of *Streptomyces tunisiensis* SA-13 revealed that shaked culture enhance the antibacterial activity than static culture, it means that both aeration and nutrient availability performed by shaking is very important for metabolic activity of this strain. Our results agreed with the findings of many researchers that found the antimicrobial productivity increased in shaking culture comparing with the static one. This is due to the fact that streptomycetes are obligate aerobic organisms [26, 31, 32].

3.7.2. Effect of Initial pH

Streptomyces tunisiensis SA-13 was seemed to produce antibacterial metabolite(s) at wide range of pH (6 -8) while the organism failed to exhibit any inhibitory effect against all tested bacterial strains at initial pH 6. The optimum initial pH value capable of promoting active metabolite biosynthesis was found to be significantly at pH 7 and 8 where the inhibition activity (14 – 15.5mm). also, at pH 7 exhibited considerable activity (13-15mm) (Fig. 5).

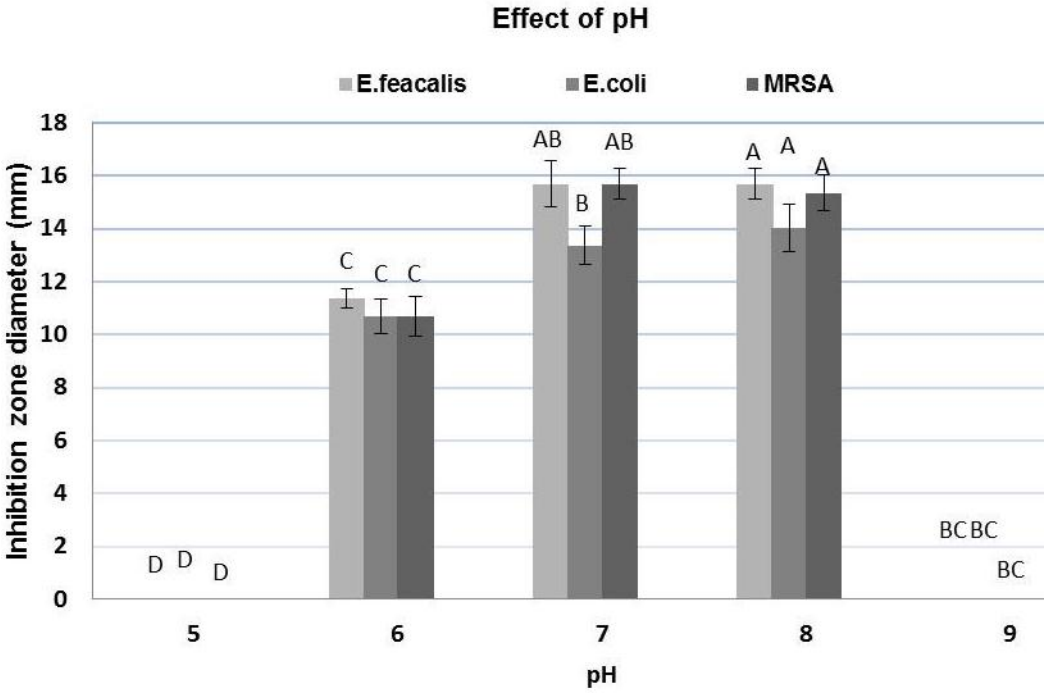

Figure-5. Effect of different pH on the antimicrobial activity of *Streptomyces tunisiensis* SA-13

The optimum initial pH of the medium is one of the key factors affecting growth, product formation of microorganisms and characters of their metabolism. Soil Actinomycetes are marked by relative intolerance of acidity usually preferring neutral or slightly alkaline pH for the growth and antagonistic activity [7]. The results obtained from this study revealed that the antibacterial activity of *Streptomyces tunisiensis* SA-13 found in pH range from 6 to 8 with optimum activity against all MDR bacteria at pH 7 and 8. This result is similar to that published by Bashir, et al. [33] that reported the antimicrobial activity increased and attained maximum with gradual increase of pH from 6 to 8 and began to decrease at pH 9. No activity was observed at pH 5 against both indicator strains and the best activity was observed at pH 7 and 8.

3.7.3. Effect of Different Culture Media

Along the screening of the best culture media for antibacterial metabolites production, the result showed that the filtrate of *Streptomyces tunisiensis* SA-13 was active against MDRB with different degrees. Riched medium was the best medium exhibiting the highest significant antibacterial activity (inhibition zone ranged from 15.6 – 17 mm) against all tested MDR bacteria (Fig. 6). So, this medium was used to determine the optimal conditions that enhance antibacterial productivity by *Streptomyces tunisiensis* SA-13.

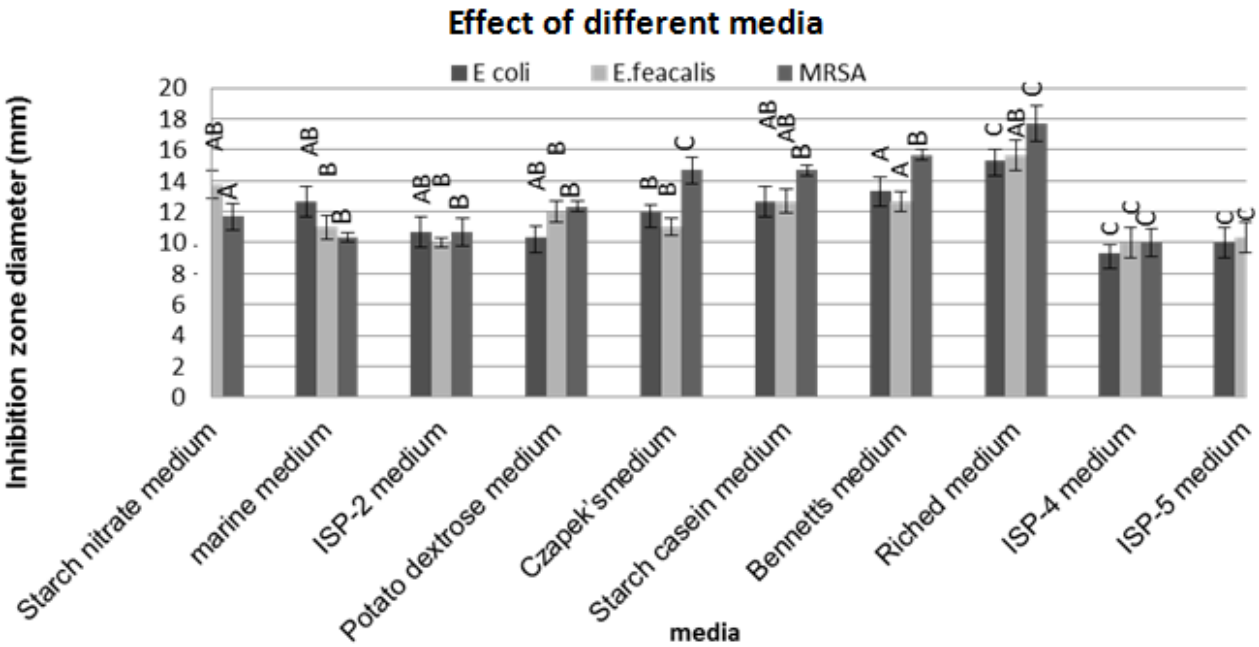

Figure-6. Effect of different culture media on the antimicrobial activity of *Streptomyces tunisiensis* SA-13

The key factors that strongly affect microbial metabolites are carbon and nitrogen sources [34]. The results showed that antimicrobial activity was higher in riched medium having sugar and peptone as carbon and nitrogen source respectively. The high yield rate from these media is not surprising because these organic substrates are the classic nutrients in Cultivation of *Streptomyces* [35].

3.7.4. Effect of Incubation Temperature

Generally, the antibacterial activity of *Streptomyces tunisiensis* SA-13 was appeared at wide range of temperature from 20 to 35℃ while the activity disappeared at 40℃. Data represented graphically in Figure 7 showed that, the optimum temperature able to produce antibacterial activity was significantly at 30℃, whereas, the diameter of inhibition zone ranged from 15 to 18mm against all tested bacteria. Also, the antibacterial activity at 20℃ was insignificantly different than 25℃ and 30℃.

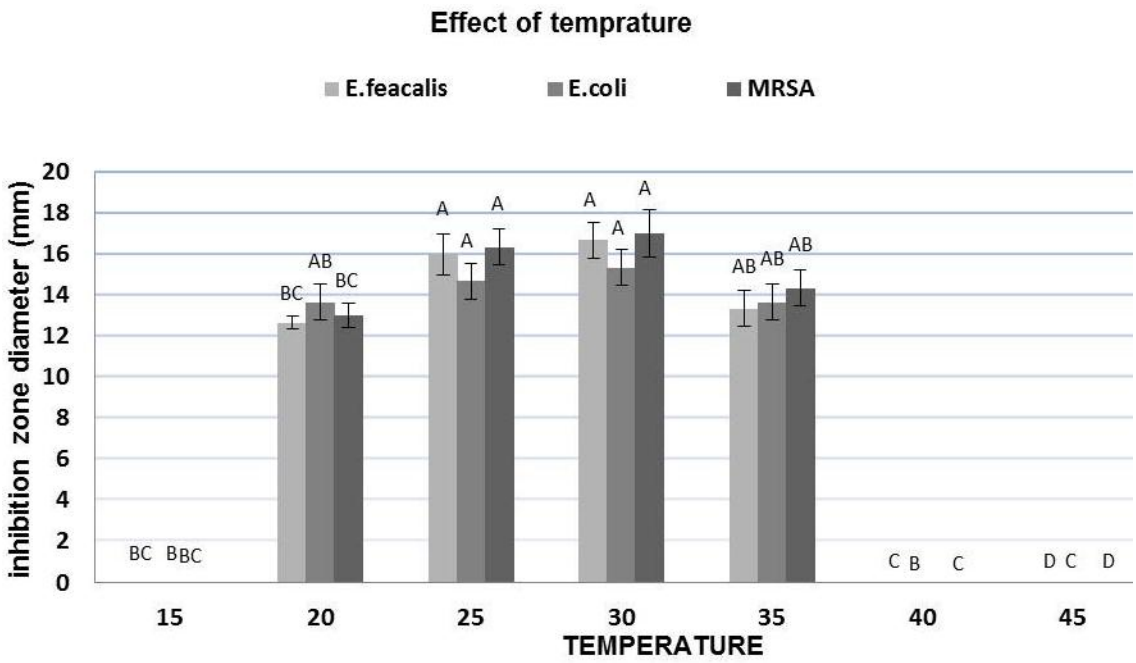

Figure-7. Effect of incubation temperature on the antimicrobial activity of *Streptomyces tunisiensis* SA-13

Temperature has direct direct effect on all metabolic process including enzyme activity and production of active metabolites [36]. The results obtained from incubation temperature study showed that, the antibacterial activity of *Streptomyces tunisiensis* SA-13 lies in the range from 20 to 35℃ with highest activity at 30℃. These results are in agreed with Abdelwahed, et al. [37] who showed that The maximum antimicrobial secondary metabolites production was obtained at incubation temperature 30°C for isolates, (*S. cyaneus* DN. 37 and *S. lavendulae* DN. 7), then obvious decline in the productivity was occurred at 40°C.

3.8. Cytotoxic Activity

Cytotoxic activity of *Streptomyces tunisiensis* SA-13 crude extract against VERO cell line was studied at different concentrations. The variation of extract activity correlated to cytotoxicity was illustrated in figure 8. The crude extract showed moderate toxicity with IC-50 200µg/ml. It was obvious there is strong correlation between antimicrobial activity and cytotoxicity. With increasing concentration, both antimicrobial activity and toxicity increase.

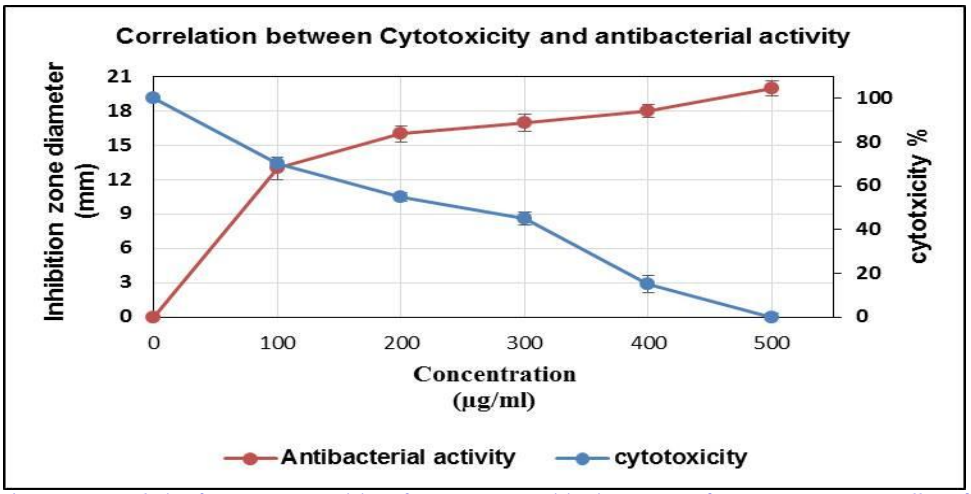

Figure-8. Correlation between cytotoxicity of *Streptomyces tunisiensis* SA-13 crude extract on VERO cells and antimicrobial activity against *Enterococcus feacalis*

4. CONCLUSION

The current study revealed that, *Streptomyces tunisiensis* SA-13 is powerful soil actinomycetes having a broad spectrum activity against resistant strains of pathogenic bacteria (ESKAPE). Further studies including separation, purification of bioactive compound and their identification may be useful for discovering of new bioactive metabolites that has activity against MDRB.

Funding: This study received no specific financial support.
Competing Interests: The author declares that there are no conflicts of interests regarding the publication of this paper.

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