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Antibacterial activity of two Streptomyces species isolated from Egyptian and Libyan soils

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Abstract

Sixty five actinomycetes isolates were collected from different Egyptian and Libyan soils. Forty eight isolates from the collected actinomycetes were found to be active against some tested pathogenic bacteria. Two different actinomycetes isolates (one from each country) which showed considerable higher antibacterial activity were identified as *Streptomyces pluricolorescens* and *Streptomyces alni.* They were also selected for optimizing the best conditions for their antibacterial activity production. The optimum incubation period, temperature and initial pH of medium for the maximum antibacterial yield were 5 days, 30ºC and pH 7, respectively, for both of *Streptomyces* species. The maximum antibacterial production was observed on Dox and starch ammonium sulfate media for *S. pluricolorescens*, while starch nitrate was the most suitable media for *S. alni* antibacterial productivity.

Keywords: Streptomyces, antibacterial activity, optimization.

Introduction

Actinomycetes are Gram-positive, free-living, saprophytic bacteria, widely distributed in soil, water, and colonizing plants. They form a distinct group of microbes as a class of their own. Early interest in actinomycetes focused mainly on their ability to yield antibiotics, along with certain vitamins and enzymes. Since the discovery of Actinomycin in 1940, the interest in the antibiotics produced by actinomycetes has been increased (Cassell and Mekalanos, 2001). From the 22,500 biologically active compounds that have been obtained from microbes, 45% are produced by actinomycetes (Bérdy, 2005). Many familiar bioactive compounds produced by actinomycetes possessed antibacterial (streptomycin, chloramphenicol, neomycin, novobiocin, nystatin, the tetracyclines, the erythromycins), antifungal (nystatin), antiviral (tunicamycin), antiparasitic (avermectin), immunosuppressive (rapamycin), antitumor (actinomycin, anthracyclines), anti-cancer (doxorubicins, daunorubicin, mitomycin, bleomycin) enzyme inhibitory (clavulanic acid) and diabetogenic (bafilomycin, streptozotocin) activities (Bérdy, 2005; Farnet and Zazopoulos, 2005). The discovery of novel bioactive

compounds continues even in the twenty first century with the addition of new antibiotics such as daptomycin, epirubicin, carbapenem analogues, and theinamycin (Sivaramkrishna and Mahajan, 2009).

The species belong to the genus *Streptomyces* constitute 50 % of the total population of soil actinomycetes (Vining, 1990; Bérdy, 1995). They are not only primarily soil inhabitants (Kuster, 1968), but also have been found widely distributed in a diverse range of aquatic ecosystem, including sediments obtained from deep sea (Walker and Colwell, 1975; Colquhoun *et al.*, 1998). They were also reported to inhibit extreme environments such as cryophilic region (Moncheva *et al.*, 2002; Raja *et al.*, 2010) and desert soil (Diraviyam *et al*., 2011).

Although thousands of antibiotics have been isolated from *Streptomyces*, these still represent a small portion of the repertoire of bioactive compounds produced (Bérdy, 1995; Watve *et al*., 2001). Therefore, isolation of new *Streptomyces* from natural resources and characterization of their secondary metabolites is a valuable endeavor.

The current study describes the isolation as many actinomycetes strains as possible from different soil samples collected from Egypt and Libya. Also, selection of the most antibacterial active strains and optimizing the conditions for maximum yield of the bioactive materials would be performed.

Materials and Methods

Soil samples collection

Soil samples were collected from different places of Egypt and Libya including rhizosphere of some inhabitant plants (Table 1) within a period of six months (February to August 2013). Soil samples were collected from various depths of the earth surface up to 1 meter depth. They were collected in sterile small plastic tubes and properly labeled with the date and location of collection. The collected soil samples were then dried and mixed with $CaCO_3$.

Isolation and purification of actinomycetes isolates

One g of each dried soil sample was suspended in 9mL sterile water, and successive serial dilutions up to 10^{-4} were prepared. An aliquot of 0.1mL of each dilution was taken and spread on 12cm Petri dishes before pouring of starch-nitrate agar medium (Waksman, 1959). 1L of the medium contains 20g Starch, 2g KNO_3, 1g K_2HPO_4, 0.5g $MgSO_4.7H_2O$, 0.5g NaCl , 3g $CaCO_3$, 20g Agar, 0.01g $FeSO_4.7H_2O$, 1.0mL trace salt solution (0.1g $FeSO_4.7H_2O$, 0.1g $MnCl_2.4H_2O$, 0.1g $ZnSO_4.7H_2O$, in100mL distilled water, pH was adjusted to 7.2). Plates were incubated at 30°C and monitored for 7 days. Growing colonies showing *Streptomyces* like appearance under light microscope were re-cultivated several times for purity isolation. The purified actinomycetes were preserved on Starch-nitrate agar slants at 4°C and in glycerol (40% v/v) at -80°C for longer periods.

Strains grouping and identification

The color of the aerial mycelia and pigment production by the isolates were determined on Starch-nitrate agar plates after 7 days of incubation at 30°C. The color of the substrate mycelia and those of the soluble pigment were determined for color grouping (Zhao *et al*., 2006). *Streptomyces* species isolated in this investigation were identified according to the International *Streptomyces* Project ISP (Shirling and Gottlieb, 1968a; 1968b; 1969; 1972; Pridham and Tresner, 1974a; 1974b; Locci, 1989).

Scanning Electron Microscopy

The spores print technique (Tresner *et al*., 1961) was used for electron microscopy examination. Grids with colloidal film were gently pressed over the sporulating surfaces of starch-nitrate agar. The grids were shadowed with chromium under vacuum before examination. The scanning electron microscopy (SEM, JEOL-100CX electron microscope at Alexandria University, Egypt) was carried out at 100K.

Screening of antibacterial activities

Antibacterial activity of the actinomycetes isolates was tested against three Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*) and five Gram- negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and Plant pathogenic *Pseudomonas* sp.). Bacterial strains (local isolates) were preserved in microbiology laboratory, Faculty of science, Damietta University, Egypt.

The bacterial suspensions were inoculated in nutrient agar medium before solidification. Then, 1 cm agar disks of 7 days old culture of each actinomycetes isolate were placed over the inoculated bacterial cultures agar plates. On the other hand, 0.1 ml of 7 days old culture metabolite (filtrated by 0.45 µm Millipore filter) of each *Streptomyces* isolate was inoculated in 1cm hole in bacterial cultures agar plates. The plates were incubated for 24hrs at 37°C, and the zones of inhibition were measured.

Antibacterial activity optimization

Types of media effect

The isolated strains (16 and 36) were cultivated on eight different media in order to study their effect on the antibacterial production. These media are starch-nitrate (Waksman, 1959), starch-ammonium sulphate, Dox, glucose-nitrate, glycerol-nitrate, glycerol-asparagine, oatmeal, yeast-malt extract (Pridham and Lyons, 1961). After incubation, the antibacterial activity was assayed as described above.

Incubation periods effect

Selected isolates were grown in liquid starch-nitrate medium, incubated at 30ºC and at 200rpm. After 3,5,7,9 and11 days, the crude metabolite filtrates were examined for their antibacterial activities.

Initial pH effect

The initial liquid starch-nitrate culture pH value was adjusted at 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10 using 1N HCl and 1N NaOH. After incubation, the final pH was recorded and the antibacterial activities of the metabolites were determined.

Temperature effect

Inoculated starch-nitrate broth media with tested isolates were incubated at 20ºC, 25ºC, 30ºC and 37ºC respectively for 5days at 200 rpm. The metabolite filtrates were further on used for antibacterial activity determination.

Results and Discussion

This study was performed to isolate and screen actinomycetes strains possessing antibacterial activities using different selective isolation medium followed by selection of the most antibacterial active strains for further investigation. Sixty five different actinomycetes were obtained mainly from the rhizosphere soil samples collected from different locations of Egypt and Libya (**Table** 1) during the year 2013. All the strains were isolated on starch-nitrate agar media which is very specific for the isolation of actinomycetes, as mostly actinomycetes are capable of degrading the polymers in this media (Demain and Davies, 1999).

All the purified isolates showed morphological characteristics of typical *Streptomyces* species, as their colonies possessed an earthy odor and were slow growing, aerobic, powdery, folded with aerial and substrate mycelia of different colors (Anderson and Wellington 2001). All of the isolated strains showed acid fast negative and Gram positive stain that fitted to the description of genus *Streptomyces*. The isolates were categorized into five color series according to their color of the mature sporulated substrate mycelium (**Table** 1 and **Figure** 1). The white series isolates were more predominant (38.5% of the total isolates). All the isolated actinomycetes strains were screened for their antibacterial activity using agar culture disc of actinomycetes on nutrient agar medium seeded with bacteria test strains (**Figure** 2). Broad spectrum antibacterial activity was observed for 73.8 % (48 out of 65) of the total tested isolates. Current results revealed higher frequency of antibacterial active strains than Denizci (1996) who found that 36% of screened 356 *Streptomyces* isolates from soils in the Aegean and East Black Sea regions of Turkey were active against tested microorganisms. The antibacterial activity of only 20 out of 150 (13.3%) actinomycetes isolates from soil samples of west of Iran was investigated (Dehnad *et al*., 2010). More recently, Laidi *et al*., (2013) reported that nineteen out of thirty five actinomycetes isolates showed noticeable antimicrobial activities and five among the nineteen were active against both Gram positive and Gram negative bacteria, yeasts and moulds. Comparing the above mentioned results with this study, we can conclude that the Egyptian and Libyan soils are rich source of actinomycetes which are metabolically active.

Table 1. Collection sites of soil samples and color grouping of the isolates.

Color of series	Isolate number	Color of aerial mycelia	Color of substrate	Cover plant	Site of soil
White	7	White	N.D	*Citrus aurantifolia*	Damietta
	9	White	pink	*Mangifera indica*	Damietta
	11	Pinkish white	pink	Plain soil	Damietta
	18	Pinkish white	pink	*Mangifera indica*	Damietta
	21	White	N.D	Plain soil	Damietta
	22	White	N.D	Plain soil	Damietta
	23	White	N.D	*Citrus slensis*	Damietta
	24	White	yellow	*Citrus slensis*	Damietta
	25	White	N.D	*Citrus slensis*	Damietta
	27	White	yellow	*Olea europea*	Benghazi
	30	White	pink	*Psidium jugave*	Benghazi
	31	White	yellow	*Morus alba*	Benghazi
	32	White	yellow	*Psidium jugave*	Benghazi
	33	White	yellow	*Prunus salicinia*	Benghazi
	37	White	yellow	*Prunus salicinia*	Benghazi
	38	White	yellow	*Prunus armeniaca*	Benghazi
	41	White	yellow	*Prunus salicinia*	Tubrok
	46	White	Dark yellow	*Citrus slensis*	Tubrok
	48	White	pink	*Psidium jugave*	Tubrok
	51	White	N.D	*Mangifera indica*	Tubrok
	52	White	yellow	*Prunus armeniaca*	Tubrok
	53	White	Gray	*Prunus salicinia*	Tubrok
	54	White	N.D	*Prunus arabica*	Tubrok
	57	White	White	*Punica granatuml*	Tubrok
	60	White	yellow	*Prunus arabica*	Benghazi
Grey	1	Gray	N.D	*Citrus aurantium*	Damietta
	3	Gray	N.D	Cairo garden	Cairo
	4	Gray	N.D	Plain soil	Damietta
	6	Gray	N.D	Plain soil	Damietta
	10	Gray	N.D	*Mangifera indica*	Cairo
	13	Gray	N.D	*Citrus aurantifoli*	Damietta
	28	Gray	N.D	*Citrus slensis*	Zleten
	29	Grayish	N.D	*Olea europea*	Benghazi
Grey	34	Gray	yellow	*Prunus armeniaca*	Tubrok
	35	Light gray	N.D	*Psidium jugave*	Benghazi
	36	Gray	Gray	*Citrus aurantium*	Benghazi
	39	Gray	Gray	*Citrus aurantium*	Benghazi
	42	Gray	N.D	*Prunus armeniaca*	Tubrok
	43	Gray	yellow	*Prunus armeniaca*	Tubrok
	44	Gray	N.D	*Punica granatuml*	Tubrok
	45	Gray	yellow	*Prunus salicinia*	Tubrok
	47	Gray	N.D	*Citrus slensis*	Tubrok
	49	Gray	N.D	*Punica granatuml*	Tubrok
	50	Gray	N.D	*Olea europea*	Tubrok
	55	Gray	N.D	*Prunus arabica*	Tubrok
	61	Gray	Brown	*Prunus arabica*	Benghazi
	63	Gray	Gray	*Morus alba*	Benghazi
	64	Gray	Gray	*Prunus arabica*	Benghazi
Yellow	5	yellow	yellow	Damietta garden	Damietta
	16	yellow	pink	*Mangifera indica*	Damietta
Red	2	Pink	Dark pink	Cairo garden	Cairo
	8	Pink	Pink	*Mangifera indica*	Damietta
	12	Pink	Dark pink	*Citrus slensis*	Damietta
	14	Pink	Pink	*Mangifera indica*	Damietta
	15	Pink	Pink	*Citrus slensis*	Damietta
	17	Pink	Dark pink	*Citrus aurantifolia*	Damietta
	19	Pink	Pink	*Mangifera indica*	Damietta
	20	Pink	N.D	*Citrus aurantifolia*	Damietta
	56	Pink	Gray	*Vitis vinifera*	Tubrok
	58	Pink	Yellow	*Prunus salicinia*	Tubrok
	59	Pink	Yellow	*Prunus arabica*	Tubrok
	62	Pink	Brown	*Prunus salicinia*	Benghazi
	65	Pink	Yellow	*Vitis vinifera*	Benghazi
Violet	26	Pink	Purple	*Morus alba*	Benghazi
	40	Light purple	Purple	*Prunus salicinia*	Tubrok

N.D = Not Detectable

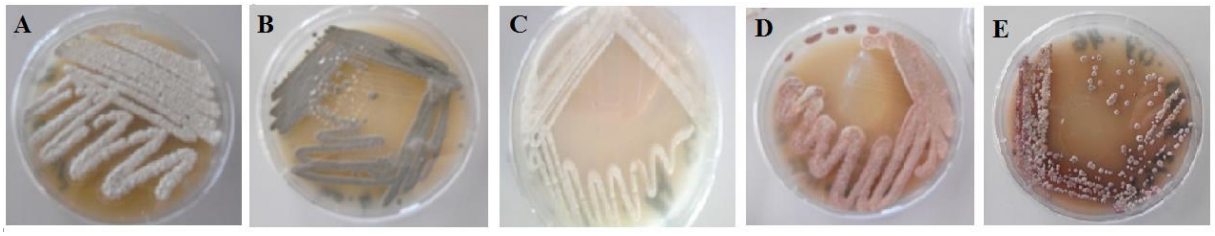

Fig 1. Representative isolates of different color series: White (A), Grey (B), Yellow (C), Red (D) and Violet (E).

The two different isolates encoded 16 and 36 showed considerable antibacterial activity towards most of the tested pathogenic bacteria than the others. They were identified as *Streptomyces pluricolorescens* (Egyptian isolate) and *Streptomyces alni* (Libyan isolate), respectively, according to their morphological and biochemical characters (**Table** 2 and 3). Also, their spores scanning ultrastructure were performed (**Figure** 3).

S. pluricolorescens was able to inhibit the growth of *B. subtilis*, *B. cereus* and *S. aureus* as Gram-positive bacteria and plant pathogenic *Pseudomonas* sp. and *P. aeruginosa* as Gram-negative bacteria. On the other hand, only *B. subtilis*, *B. cereus*, *K. pneumoniae* and *Pseudomonas sp.* were inhibited by *S. alni*. When Laidi *et al.*, (2013) tested their isolates, they documented that *S. labedae* strain RAF-11 exhibited inhibition zone against *B. subtilis*, *M. luteus*, *S. aureus*, *E. coli* and *K. pneumoniae*. Also in accordance to our result, Eleven Egyptian bioactive actinomycetes isolate (El-Shobaky 2010) and other eighteen isolates (Gaber 2011)

produced antagonistic metabolites against wide range of bacteria.

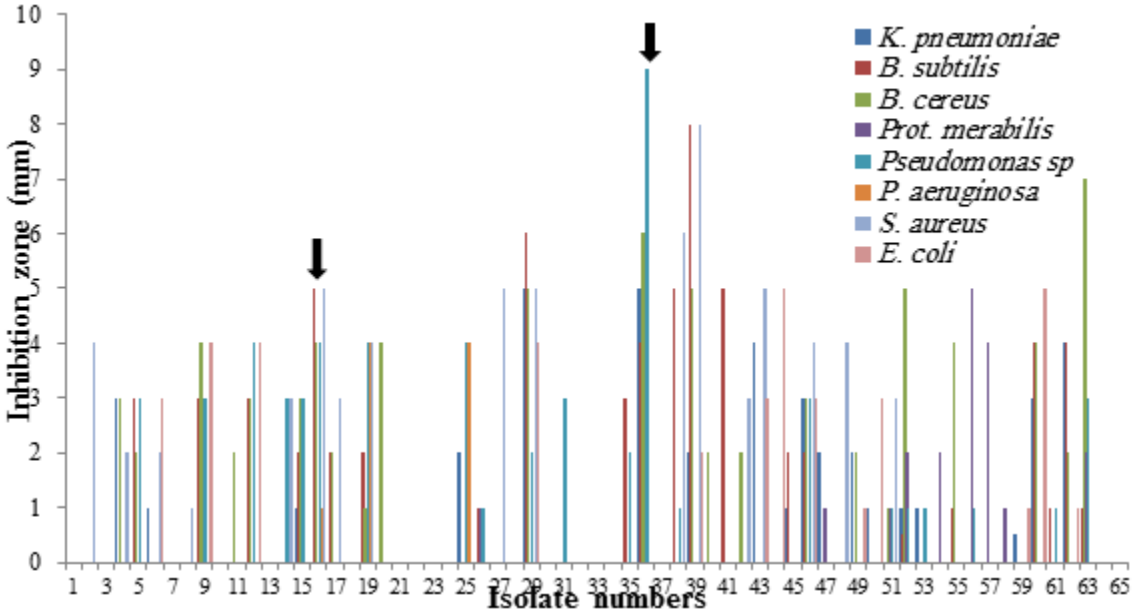

Fig 2. Antibacterial activity of the 65 actinomycetes isolates against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirablis*, Plant pathogenic *pseudomonas sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The arrows indicated the selected isolates (16 and 36)

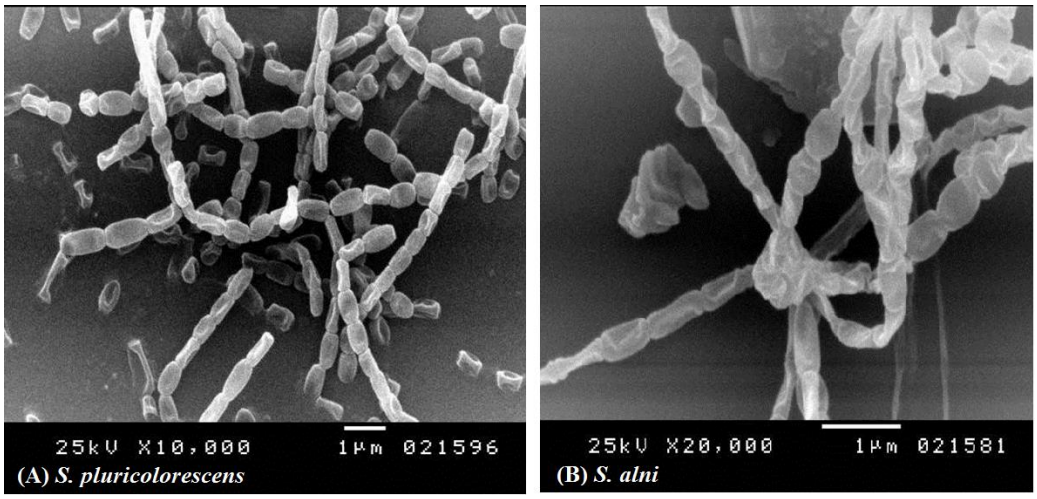

Fig 3. The scanning electron microscopy showing smooth spore surface of *S. pluricolorescens* (A) and *S. alni* (B)

Table 2. Cultural properties of seven days old cultures of *S. pluricolorescens* and *S. alni* strains on different media

Type of Medium	Growth appearance		Colour Of * Aerial mycelium		Substrate mycelium		Pigment		Growth intensity	
	S. pluricolorescens	*S. alni*	*S. pluricolorescens*	*S. alni*	*S. pluricolorescens*	*S. alni*	*S. pluricolorescens*	*S. alni*	*S. pluricolorescens*	*S. alni*
Starch nitrate	Powdery	Powdery	Yellow	Grey	Pale-brown	Pale- grey	Slight brown	No pigment	++	++
Starch amm. sulphate	Powdery	Powdery	Yellow	Grey	Pale-pink	Pale- grey	No pigment	No pigment	++	++
Dox	Powdery	Powdery	Yellow	Grey	Pale-pink	Pale- grey	No pigment	No pigment	++	++
Glucose nitrate	Powdery	Powdery	Yellow	Grey	Yellow	Pale- grey	No pigment	No pigment	++	++
Glycerol nitrate	Powdery	Powdery	White	Grey	White	Pale- grey	No pigment	No pigment	+	++
Glycerol aspargin	Powdery	Powdery	Yellow	Grey	White	Pale- grey	No pigment	No pigment	+	++
Oatmeal	Powdery	Powdery	Page-cream	Grey	Pale-brown	Pale- grey	Slight brown	No pigment	++	++
Yeast malt	Powdery	Waxy	Pale-pink	White	Pale-pink	White	No pigment	No pigment	±	++

* The colour of substrate mycelium and medium was not pH sensitive when treated with 0.05N NaOH or 0.05N HCl. (++) good, (+) moderate, (±) poor.

Table 3. Cultural, morphological and physiological characteristics of *S. pluricolorescens* and *S. alni*

Characters		*S. pluricolorescens*	*S. pluricolorescens* type strain	*S. alni*	*S. alni* type strain
Colour and pigmentation	Aerial mass color	Yellow	Yellow	Pale grey	Pale grey
	Melanoid pigment on: tyrosine, peptone yeast and synthetic media	-	-	-	-
	Reverse side pigment	Pale reddish to yellow	Pale reddish to yellow	Grey	Grey
	Soluble pigment	-	-	-	-
Spore morphology	Spore chain	Straight	Rectiflexibiles	Straight to flexuous	Straight to flexuous
	Spore surface	Smooth	Smooth	Smooth	Smooth
Carbon source utilization	Arabinose	-	±	+	+
	Xylose	+	+	+	+
	Inositol	+	-	+	±
	Mannitol	+	+	+	+
	Fructose	+	+	+	+
	Rhamnose	+	+	-	±
	Sucrose	+	-	+	±
	Raffinose	+	-	+	±
Nitrogen source utilization	Potassium nitrate	++	nd	+	nd
	L-valine	±	nd	+	nd
	L-threonine	+	nd	-	nd
	L-serine	+	nd	+	nd
	L-Methionine	+	nd	-	nd
	L-histidine	+	nd	+	nd
	Hydroxy proline	±	nd	++	nd
	L-proline	++	nd	++	nd
	L-Tyrosine	+	nd	-	nd
	Casein	+	nd	-	nd
	Peptone	++	nd	++	nd
	Arginine	+		+	
Physiological properties	Milk coagulation	±	nd	+	nd
	Milk peptonization	±	nd	+	nd
	Starch hydrolysis	+	nd	+	nd
	Urea hydrolysis	+	nd	+	nd
	Gelatin liquefaction	+	nd	+	nd
	Melanin/L-tyrosine	-	nd	-	nd
	Cellulose degradation	-	nd	-	nd
	Esculin degradation	+	nd	+	nd

(++) good, (+) moderate, (±) poor, (-) nil, (nd) not detected

The optimum condition for growing the two isolates was studied. The temperature range for growing was 30ºC to 37ºC for *S. pluricolorescens* **(Figure 4)**, while *S. alni* was able to grow at 20ºC to 40ºC (**Figure 5**). The growth of both species was not detected below or above those temperatures when tested on starch nitrate medium. The difference in their mesophilic character might be attributed to the effect of geographic area of isolation site, as *S. alni* was isolated from Benghazi (Libya) which possesses climatic characters different from Damietta (Egypt) for *S. pluricolorescens*. The optimum conditions for maximum antibacterial production were observed at 30ºC of temperature, pH7 and maximum period of culturing reached 5 days for both *S. pluricolorescens* and *S. alni* (data not shown). Hassan *et al*., (2001) studied the effect of temperature on the antimicrobial productivity produced by *S. violatus* and they found that increasing the incubation temperature from 20°C to 30°C led to increase growth and productivity of the antibiotic, while raising the temperature higher than 35°C has had an adverse effect on growth and productivity. Maximum yield of the active metabolite produced by *S. pluricolorescens* and *S.*

alni was obtained at of 30°C which is quite similar to some other mesophilic *Streptomyces* species and isolates (Gubte and Kulkarni, 2002; Al-Khaldi, 2003; Kiviharju *et al*., 2004; Atta *et al*., 2011; Vijayakumar *et al*., 2012; Ababutain *et al*., 2013; Bhavana *et al*., 2014). Also, other study by Padma *et al*., (2002) and Jain *et al*., (2011) recorded that the best incubation temperature was 29°C and 28°C respectively. Some actinomycetes strains might need higher temperature up to 35°C to get the maximum antibiotic production as in case of *S. albidoflavus* (Narayana and Vijayalakshmi 2008).

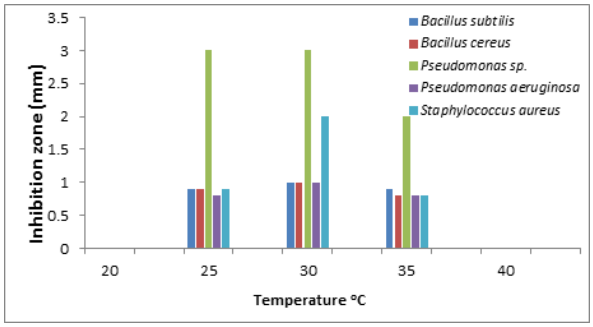

Fig 4. Effect of temperature ^{o}C on the antibacterial activity of *S. pluricolorescens* against different bacterial strains using 100 µl filtrate of *S. pluricolorescens* culture.

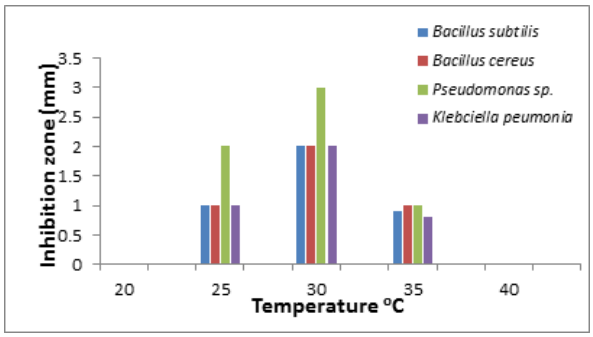

Fig 5. Effect of temperature ^{o}C on the antibacterial activity of *S. alni* against different bacterial strains using 100 µl filtrate of *S. alni* culture.

Maximum antibacterial activity was obtained in case of both tested strains at an initial pH 7 (**Figures 6 and 7**). Chattopadhyay and Sen (1997) noted that pH 7 is the most appropriate pH values to produce the highest amount of antibiotic of different types of *Streptomyces*. This is also in agreement with Narayana and Vijayalakshmi (2008); Atta *et al*., (2011) and Vijayakumar *et al*., (2012). Furthermore, It was found that the highest amount of productivity of antibiotic by *S. violatus* and *S. carpaticus* was at pH 7.5 and 7.2 respectively (El-Naggar *et al*., 2003; Bhavana *et al*., 2014). Also other studies by Crawford *et al*., (1993) and Vijayakumar *et al*., (2012) recorded that the best growth of actinomycetes strains were between pH 6.5-8 and few could not grow at pH 6.0, nevertheless the failure of a large number of actinomycetes to grow at pH 5.5. On the contrary, Holtzel *et al*., (1998) and Ababutain *et al*., (2013) reported the highest antibiotic production at pH 5.5 and 6 respectively.

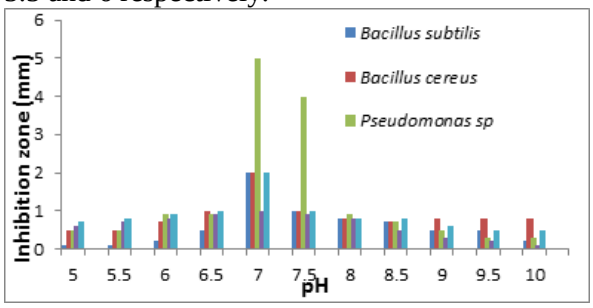

Fig 6. Effect of pH on the antibacterial activity of *S. pluricolorescens* against different bacterial strains using 100 µl filtrate of *S. pluricolorescens* culture.

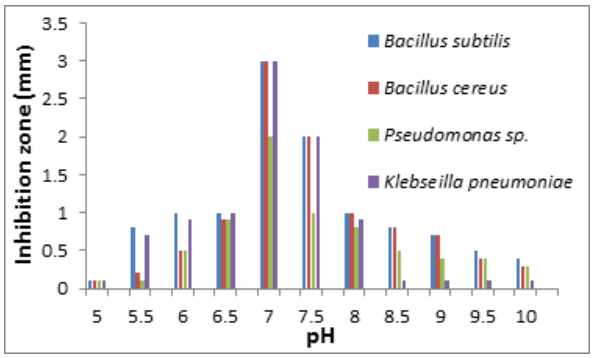

Fig 7. Effect of pH on the antibacterial activity of *S. alni* against different bacterial strains using 100 µl filtrate of *S. alni* culture.

Maximum growth and biosynthesis occurred at the end of an incubation period of five days on Starch nitrate medium for both studied strains (**Figures 8 and 9**). Five days incubation period was found to be the best by many researchers (Ryoo *et al*., 1997; Holtzel *et al*., 1998; Narayana and Vijayalakshmi 2008; Vijayakumar *et al*., 2012; Bhavana *et al*., 2014). In addition, the highest productivity after four days of incubation was also recorded (El-Naggar *et al*., 2003; Atta *et al*., 2011; Song *et al*., 2012). Some *Streptomyses* species needed more incubation period for maximum bioactive compounds production reached up to seven days (Hassan *et al*., 2001; Venkateswarlu *et al*., 2004; Al-Zahrani 2007; Ababutain *et al*., 2013) and even ten days (Jain *et al*., 2011).

The best production of antibacterial compounds for *S. pluricolorescens* and *S. alni* was optimized on eight different types of media. Both agar discs and Millipore-sterile filtrates of each solid and

culture growth medium, respectively, were tested against bacteria (**Figures** 10 **and 11**). The results showed that the most suitable media for *S. pluricolorescens* antibacterial activity were Dox (containing sucrose and sodium nitrate) and starch ammonium sulfate media followed by starch nitrate medium, while starch-nitrate medium exhibited the maximum antibacterial activity for *S. alni*. In general, many researchers found that starch nitrate medium is suitable for best productivity (Gaber 2011; Vijayakumar *et al*., 2012; Ababutain *et al*., 2013), as the soluble starch is considered best carbon source for the highest productivity of the bioactive substances (Atta *et al*., 2011; Vijayakumar *et al*., 2012). Different carbon sources could influence the maximum productivity depending on the *Streptomyces* species. For example, glycerol was the most suitable carbon source for *S. antibioticus* productivity (Haque *et al*., 1995), and maltose for *S. albidoflavus*. Sometime, as in the case of Actinomycetes YJ1 strain; a mixture of two carbon sources like sucrose and soluble starch is most appropriate (Song *et al*., 2012).

Regarding to the nitrogen sources, it was found that sodium nitrate (Aman 2001; El-Naggar 2003), in addition, potassium nitrate (Atta *et al*., 2011) were the most appropriate source of nitrogen for best productivity. Sometime ammonium as succinate salt possessed a good productivity (Gesheva *et al*., (2004). Venkateswarlu *et al*., (2004) recorded that the rifamycin productivity was increased to the maximum when ammonium sulphate, soybean and peanuts were used. Amino acids such as lysine also increased the production of antibiotic extracted from *S. antibioticus* (Theobald *et al*., 2000).

Further investigations should be carried out in order to separate and identify probably novel antibiotic active compounds from these promising *Streptomyces* isolates.

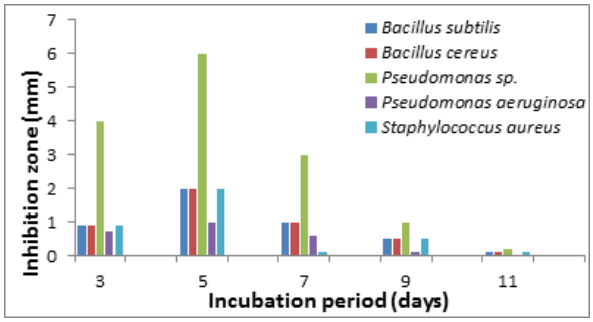

Fig 8. Effect of incubation period on the antibacterial activity of *S. pluricolorescens* against different bacterial strains using 100 µl filtrate of *S. pluricolorescens* culture.

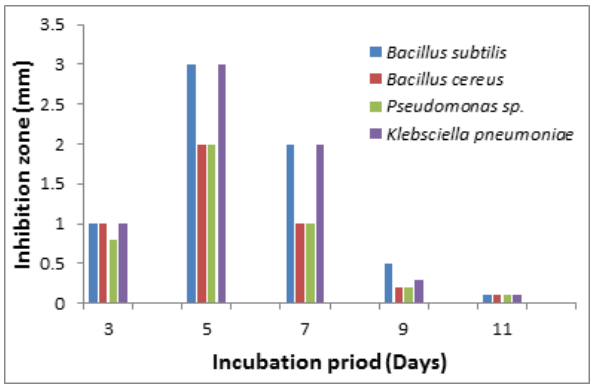

Fig 9. Effect of incubation period on the antibacterial activity of *S. alni* against different bacterial strains using 100 µl filtrate of *S. alni* culture.

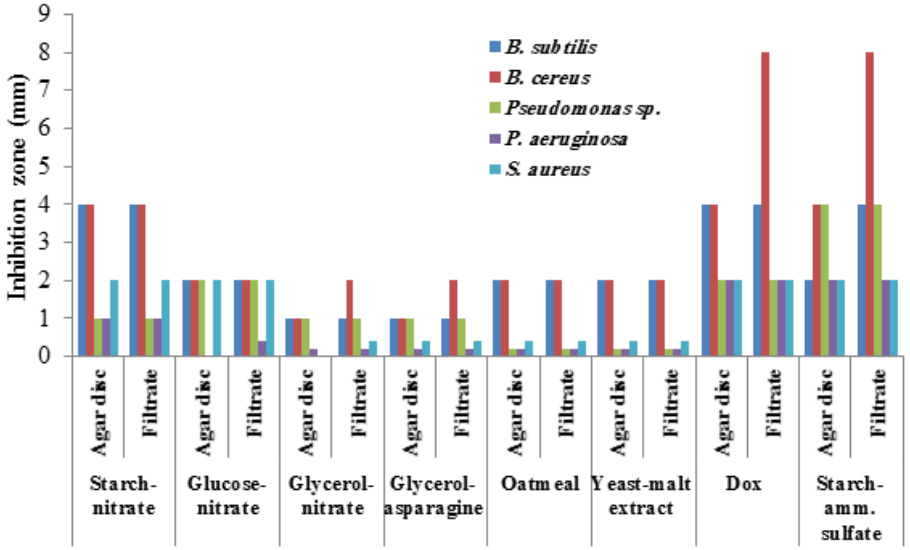

Fig 10. Effect of the type of media on the antibacterial activity of *S. pluricolorescens* against different bacterial strains using 1cm agar disc and 100 µl filtrate of *S. pluricolorescens* culture.

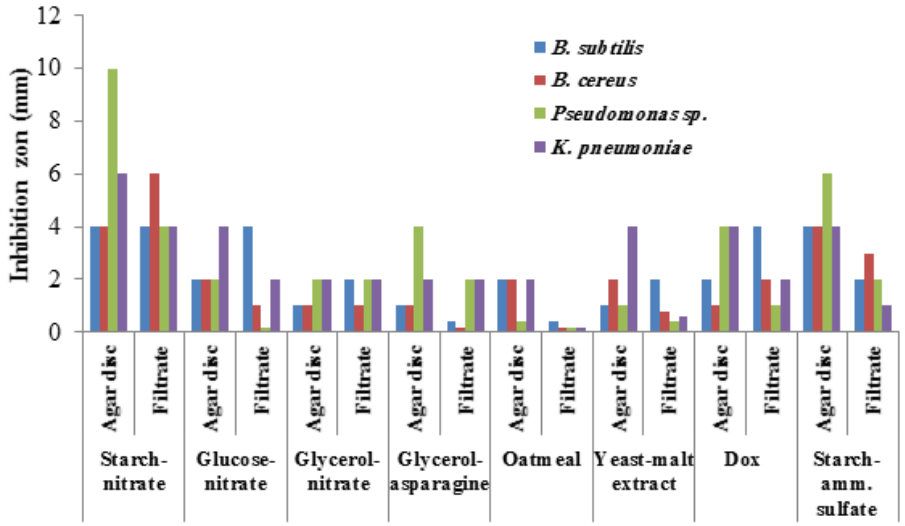

Fig 11. Effect of the type of media on the antibacterial activity *of S. alni* against different bacterial strains using 1cm agar disc and 100 µl filtrate of *S. alni* culture.

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الملخص العربي

عنوان البحث: النشاط ضد بكتيري لنوعين من جنس إستربتوميسيس معزولتين من تربتين مصرية وليبية

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تهدف الدراسة لتعريف الأنشطة الضد بكتيرية لخمسة وستون عزلة من الأكتينوميسيتات المعزولة من التربة المصرية والليبية. أظهرت ثمانية وأربعون عزلة منهم نشاطا مضادا للبكتيريا بإختبارها ضد بعض سلالات البكتيريا (٣ موجبة لصبغة جرام و ٥ سالبة لصبغة جرام) . تم إختيار أفضل عزلتين أظهرا نشاطا ضد بكتيرى (واحدة من كل دولة) واللتان تم تعريفهما وتسميتهما إستربتوميسيس بلوريكولورسينس وإستربتوميسيس ألنى. كم تم إختبار الظروف المثلى لإنتاج المضادات البكتيرية لهما. كانت أنسب فترة زمنية للإنتاج هى بعد خمس أيام من النمو، ودرجة حرارة النمو المثلى كانت ٣٠ م° ، أما درجة ٧ للأس الهيدروجينى هى الأفضل. تم أيضا دراسة هذه العزلات من حيث قدرتها على إنتاج المواد الضد بكتيرية عند تنميتها على أوساط غذائية مناسبة. ولقد تم تنميتها على ثمانية أوساط غذائية مختلفة هم: نشا-نترات، جلوكوز-نترات، جليسرول-نترات، جليسرول-أسبراجين، الشوفان، مستخلص الخميرة-الشعير، دوكس، نشا-كبريتات الأمونيوم. أظهرت النتائج أن أفضل الأوساط الغذائية لإنتاج المواد الضد بكتيرية للعزلة إستربتوميسيس بلوريكولورسينس كان الوسط دوكس يليه نشا-نترات. وكان الوسط الغذائى نشا-نترات الأفضل في حالة العزلة إستربتوميسيس ألنى.