Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22, No. (3) : 2020 : 395-404

ISSN-0972-3005

OPTIMAL CONDITIONS, EXTRACTION, AND PARTIAL PURIFICATION OF A POTENTIAL NOVEL ANTIMICROBIAL COMPOUND PRODUCED BY *STREPTOMYCES* N-404 STRAIN

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(Received 15 February, 2020; accepted 23 March, 2020)

Key words : *Bioactive, Inhibitory, Purification, Multi-resistant, Streptomyces*

Abstract – In this study the optimal conditions in addition to extraction, partial purification and characterization of antimicrobial compound produced by *Streptomyces* N-404 strain were determined. Results showed that *Streptomyces* N-404 strain optimally produced the antimicrobial compound against the test organisms after 5 days of incubation in nutrient broth at 28 ºC and initial pH of 8.5. Analysis of the cell free broth revealed that it contains an antimicrobial compound which was stable over a wide range of pHs and temperatures, and retained inhibitory activity after heating and treatment with different enzymes. The minimum inhibitory concentration (MIC) of the water soluble of concentrated ethanolic crude extract against *Staphylococcus aureus* and *Escherichia coli* was 5 and 10 mg/mL, respectively. Purification experiments for the active compound out of permutite column by a gradient solution of KOH revealed the elution of one major peak and a 74% increase in activity against *S. aureus* with a maximum inhibition zone diameter of 33 mm. When *Streptomyces* N-404 strain was tested for its sensitivity to gentamycin and kanamycin and the partial purified active compound was compared to these antibiotics, data indicated that this strain is not producing these 2 antibiotics, and the active compound resemble them in their temperature and pH stability, but differs from these compounds in TLC pattern, adsorption to charcoal under basic and acidic conditions, and UV spectrum. These findings stress the novelty of the inhibitory compound produced by *Streptomyces* N-404 strain.

INTRODUCTION

An important obstacle to the long-term treatment of an antimicrobial agent is the appearance and spread of resistance to the agent. The development of resistance to commonly used antibiotics is of particular concern when it occurs in pathogens that cause invasive disease. For this reason, intensive screening programs for antibiotics are still running in different parts of the world and mainly from genus *Streptomyces* which provide a rich source of antibiotics (Goodfellow and O'Donnell 1989; Takizawa *et al*., 1993; Saadoun *et al*., 1999; Saadoun and Al-Momani 2000; Saadoun *et al*., 2000; Saadoun and Gharaibeh 2003; Saadoun *et al*., 2008; Saadoun *et al*., 2013, 2015). The traditional approach to isolation has been the use of terrestrial soils, which provide a rich source of these microorganisms (Labeda and Shearer 1990) with screening programs were and still being initiated in various countries for isolation such antibiotic-producing *Streptomyces* strains mainly from soil samples. However, due to extensive use of these antibiotics as chemotherapeutic agents, they have provided an environment conducive to the selection of bacterial strains resistant to currently available antibiotics particularly in the hospital settings. Thus there remains a need for new sources and strategies to develop antimicrobial agents that combine broad spectrum of activity with resistance to inactivation by bacterial enzymes. Therefore, one of the approaches that can be followed is to expand the screening activity of the members of genus *Streptomyces* against test pathogenic bacteria or fungi

that are resistant to several known antibiotics.

Saadoun *et al*. (1999) previously reported a total of 29 out of 90 soil *Streptomyces* isolates have the ability to inhibit tested Gram negative and positive bacteria in addition to *Candida albicans*, with the ability of the isolate N-404 to inhibit all of the tested pathogens. In the present investigation, the *Streptomyces* N-404 strain was further studied to determine the optimal conditions for antibiotic production; extract and purify the active substance(s) being produced by this active strain using different extraction and chromatographic techniques; and to study the stability of the active produced substance(s) under the effect of different temperatures, pH and digestive enzymes (amylase, pepsin and trypsin).

MATERIALS AND METHODS

Isolation and characterization of *Streptomyces* sp.

Streptomyces N-404 strain was previously isolated and characterized from Jordanian soils with its ability to inhibit several multi-drug resistant Gram positive and negative pathogens in addition to *Candida albicans* (Saadoun *et al*., 1999).

Preparation of tester strains

Escherichia coli and *Staphyloccus* sp. bacterial pathogens previously used by Saadoun *et al*., (1999) and reported to be sensitive or resistant to different antibiotics that are commonly used in hospitals were used in this study.

Evaluation of Streptomyces N-404 strain to produce antimicrobial compounds in different broth cultures against tester strains

Streptomyces N-404 strain was grown in different submerged cultures in 250 mL flasks containing 50 mL of broth culture media: Nutrient Broth (NB), Starch–Casein- Nitrate Broth (SCNB), Oatmeal Broth (OM), Inorganic Salt Starch Broth (ISSB), and Yeast Extract Malt Extract Broth (YEMEB). A seed culture was prepared by growing the *Streptomyces* N-404 strain on oatmeal agar at 28 °C for 7 days. After growth, the whole aerial mycelium was scrapped by a loop then suspended in 10 mL of sterile distilled water. Flasks were inoculated with 1 mL aliquotes of active *Streptomyces* spore suspension and incubated at 28 °C ± 1 with shaking in a rotary shaker incubator (TEQ, Portugal) at 100 rpm for 21 days. After growth, 1 mL of culture broth was aseptically withdrawn at 3, 5, 7, 10, 12, 14, 17, 19, and 21 days intervals and transferred to a sterile tube then centrifuged at 4000 g for 10 min.

Tester organisms were activated by inoculation into Mueller-Hinton broth or trypticase soy broth (Oxoid, UK) then incubated at 37 °C for 24 h. Turbidity of organisms in the broth was adjusted to be equal to or greater than 0.5 McFarland turbidity standards (1.5×10^8 cfu/mL). The tester organisms were homogeneously inoculated by a sterile cotton swab on the surface of a freshly prepared Mueller-Hinton (Oxoid, UK) agar plates. Three cores of 6 mm diameter were removed from the Mueller Hinton agar then filled up with 50-70 µL of the supernatant culture broth (Hugo and Russel 1977). Plates were incubated at 37 °C ± 1 for 24 h then inhibition zones were visualized and recorded.

Optimal temperature conditions for antibiotic production

This was determined by growing *Streptomyces* N-404 strain in submerged cultures in 250 mL flasks containing 50 mL of the best broth culture media that gave the best inhibition zones against the tester pathogens. Flasks were inoculated as describes before and incubated at 20, 25, 28, 30, and 37 °C with shaking at 100 rpm for 10 days. The 28 °C ±1 was used a control. Inhibition zones were determined as before after 5 days by agar hole diffusion method against the tester pathogens.

Optimal pH conditions for antibiotic production

After selection of the best medium for antimicrobial production, the pH of this medium (Nutrient Broth, NB) was adjusted to the values of 5.0, 5.5, 6.0, 6.5, 7.0, 7.2, 7.5, 8.0, 8.5, and 9 by using Corning pH meter that was calibrated each time by 1M HCl and 1M NaOH. pH 7.2 was used as a control. Flasks of 250 ml containing 50 mL of NB were inoculated with *Streptomyces* N-404 strain as before and incubated with shaking at 100 rpm for 5 days. Inhibition zones were determined as before after 5 days by agar hole diffusion method against the tester pathogens.

Effect of temperature and pH on stability of the antimicrobial compound

Cell free broth culture of *Streptomyces* N-404 strain grown under the optimal conditions (28 °C and pH 8.5 for 5 days) was heated to 62, 85, and 100 °C for 30, 15, and 10 min, respectively, and also after autoclaving at 121 °C for 15 min. Broth cultures were cooled to room temperature before testing for

activity The stability of the substance responsible for the inhibition was also studied after incubation at 4 °C for 7 days. The activity was also evaluated by modifying the pH of the supernatants between 2.5 and 12 with 2 N NaOH and 1 N HCl. After temperature and pH treatments, broth cultures were tested for activity as described before.

Effect of digestive enzymes on stability of the antimicrobial compound

Cell free broth culture of *Streptomyces* N-404 strain grown under the optimal conditions (28 °C and pH 8.5 for 5 days) was tested for sensitivity to digestive enzymes by incubating the cell-free broth with different enzymes: trypsin, amylase, proteinase-K and lysozyme (Across, Belgium). All enzymes were prepared as 10 mg/mL of 0.1 M Na-Phosphate buffer, pH 7.4. Cell-free culture broth/enzyme mixtures were incubated for 2 hr at 37 °C except for proteinase-K which was incubated at 55 °C. Inhibition zones were determined as before after 5 days by agar hole diffusion method against the tester pathogens.

Production of the antimicrobial compound by Streptomyces N-404 strain in a large scale

Large scale fermentation was carried out under the above optimal conditions using ten 1L Erlenmeyer flasks containing 200 mL of NB, and a total of two liters was prepared. Inoculation with the active *Streptomyces* N-404 strain spore suspension and incubation were done as described before. The activity of *Streptomyces* N-404 strain against the tester pathogens was repeated to confirm its production of the inhibitory compounds after large scale and optimal conditions. Inhibition zones were determined as before after 5 days by agar hole diffusion method against the tester pathogens.

Partial isolation and purification of the antmicrobial compound

After fermentation under the optimal conditions to produce the antimicrobial compound, the cell-free culture broth from each flask was separated from the mycelium by centrifugation at 4000 rpm for 15 min at 4 °C. The separated whole broth was extracted by mixing with absolute ethanol (4:1 v/v), then vigorously shaken and allowed to separate for overnight at 4 °C. After extraction, the aquous lower layer was discarded and the supernatant was concentrated under reduced pressure in a vacuum rotary pump (Heidolph, Germany) until no solvent was left. The dry weight of the concentrated ethanolic extract was determined then suspended in 40 mL of sterile distilled water to have a concentration of 100 mg/mL. Aliquotes of 50-70 µL of the water soluble extracted material were placed in 6 mm holes made in Mueller Hinton agar medium that has been previously seeded with the tested pathogens. Plates were incubated at 37 °C ± 1 for 24 h and examined for zones of inhibition (Malik 1997).

Determination of minimum inhibitory concentration of Streptomyces N-404 strain against the tester pathogens

The minimum inhibitory concentration of *Streptomyces* N-404 strain concentrated ethanolic extract was evaluated using the tube dilution assay according to Philips *et al.* (1991) and starting with a concentration of 10 mg/mL. The activity of the concentrated ethanolic extract against the different tester pathogens was compared to the activity of the cell-free broth production medium.

Partial purification of the concentrated ethanolic extract of *Streptomyces* N-404 strain

Purification of the antimicrobial compound by different exchangers

Different cationic and anionic exchangers in addition to adsorbents (Table 2) were tested to determine which exchanger is the best for further purification. Seventy five ml of NB containing the antimicrobial compound were adjusted to pH 5.0 and 9.0 then 0.5 mL of the adjusted broth was mixed with 100 mg of cationic and anionic exchangers, respectively. Tubes were mixed by vortixing vigorously for 15 min, and then centrifuged at 5000 rpm for 2 min. The activity of the supernatants of each cationic, anionic and adsorbents was tested for activity as described before against *S. aureus* only.

Purification of the antimicrobial compound by permutite resins

A permutite adsorbent resin was prepared by washing with distilled water for overnight, dried at room temperature then packed in a 1 x 40 cm glass column. One mL of the water soluble of the concentrated ethanolic crude extract (100 mg/mL) was loaded to this column then eluted with a gradient solution of 0.05 M KOH. Fractions of 2 mL/tube were collected by a fraction collector (Spectrum™ Spectra/Chrom™, Fisher Scientific) with a flow rate of 1 mL/7 min. The activity of each

fraction was tested against *S. aureus* and those that showed similar activity were pooled together for further antimicrobial analysis.

Removal of KOH from the purified antimicrobial compound

In order to remove the KOH residues from the active compound, 2 mL of the active fractions were reacted with 0.1 mL of 1M $HCLO_4$ according to the following reaction:

KOH + $HCLO_4$! $KCLO_4$ (ppt) + H_2O. Tubes were incubated in the refrigerator for overnight, then centrifuged at 5000 rpm for 1 min, and then the supernatant was transferred to another clean tube. The activity of the fractions was further tested against *S. aureus*.

Chromatography of the antimicrobial compound

The concentrated ethanolic extract was analyzed by thin layer chromatography (TLC) according to the method of Batrakov *et al.* (2003) with slight modifications. Briefly, the samples in addition to two standard antibiotics (gentamycin and kanamycin) were spotted on to 2×10 cm silica gel TLC plates (Merck, KgaA, Germany; 60 F254, 0.25 mm) using capillary glass tubes about 2 cm above the bottom of the plates. The plates were placed in a chromatography jar containing mixture of butanol, acetic acid, and water (4:1:1 v/v). Then the plates were left in the solvent and incubated at room temperature until the solvent moved across the plate from bottom to top. The plates were removed from the jar, allowed to dry, and then visualized under ultraviolet irradiations at 254 nm and R_f values were determined.

Scanning of the antimicrobial compound at UV region

The absorption spectra of the permutite pooled fractions and concentrated ethanolic extract in addition to the standard antibiotics (gentamycin and kanamycin) were determined in the U.V region (200-400 nm) by using a U.V visible spectrophotometer (Beckman DU, Germany) (Lee and Hwang 2002).

Comparison of the active substance with gentamycin and kanamycin

The antimicrobial compound was compared with known antibiotics (gentamycin and kanamycin) according to temperature and pH stability; adsorption to charcoal granules at pH 6 and 9; TLC pattern and UV spectrum properties.

Sensitivity of Streptomyces N-404 strain to gentamycin and kanamycin

To demonstrate that *Streptomyces* N-404 strain is not producing gentamycin and kanamycin antibiotics, the susceptibility to gentamycin (10 mg) and kanamycin (10 mg) was determined disc agar diffusion method (Bauer *et al*. 1966)

RESULTS AND DISCUSSION

In a previous study, Saadoun *et al*. (1999) reported the isolation and characterization of 90 different *Streptomyces* isolates. They tested the antibiotic activity of the recovered isolates against multi-drug resistant bacteria and their results indicated that only 18 isolates were able to inhibit one or more of these pathogens. Overall, data showed that *Streptomyces* N-404 strain was able to inhibit all of the tester pathogens with a maximum inhibition zone diameter ranged between 9 and 12 mm.

After comparing the antibiotic activity of N-404 strain with 22 standard antibiotics commonly used in hospitals , data indicated that this strain does not produce the following antibiotics: GN10: Gentamycin; AM10: Amoxicillin; STR: Streptomycin; OFF: Offloxacin (Novecin); COT: Cotimoxazole; ERY: Erythromycin; OXC: Oxaxillin; PEN: Penicillin; VAN: Vancomycin; CLT: Cephaloythin; AUG: Augmantin; CTX: Cefotaxime; TCP: Teicoplanin; TET: Tetracycline; CMD: Cefamandole; CHL: Chloramphenicol; TOB: Tobramycin; CPR: Ciprofloxacin; SXT: Sulphamethoxazole; IMP: Impecillin; CB100: Carbenicillin. However, this isolate may have the ability to produce CTX (Cefotaxime) and CXM (Cefuroxime sodium) antibiotics or new antibiotic(s) other than CTX and CXM. After comparing the antibiotic activity of the N-404 strain with the activity of CTX and CXM, data indicated that N- 404 strain is producing inhibitory active compounds other than CTX and CXM. The activity of the *Streptomyces* N-404 strain stressed its potential as antagonists against several medical pathogens.

Optimal condition for production of antimicrobial compound by Streptomyces N-404 strain

To find the optimal conditions for *Streptomyces* N-404 strain growth and antimicrobial production, the effect of culture media, incubation temperature and time, and initial pH were investigated. Figure 1

shows that nutrient broth (NB) medium favors the production of the antimicrobial compound by *Streptomyces* N-404 strain after 5 days of incubation with the ability of begining of production after 48 h and maximizing after 5 days (Fig. 1d). This optimal production was in parrallel with the minimal incubation time, highets inhibition zones and activity against both tester pathogens. However, the production was different when *Streptomyces* N-404 strain was grown in the other culture media (Fig. 1a,c,e). Therefore, further fermentation experiments have been performed in NB medium in order to

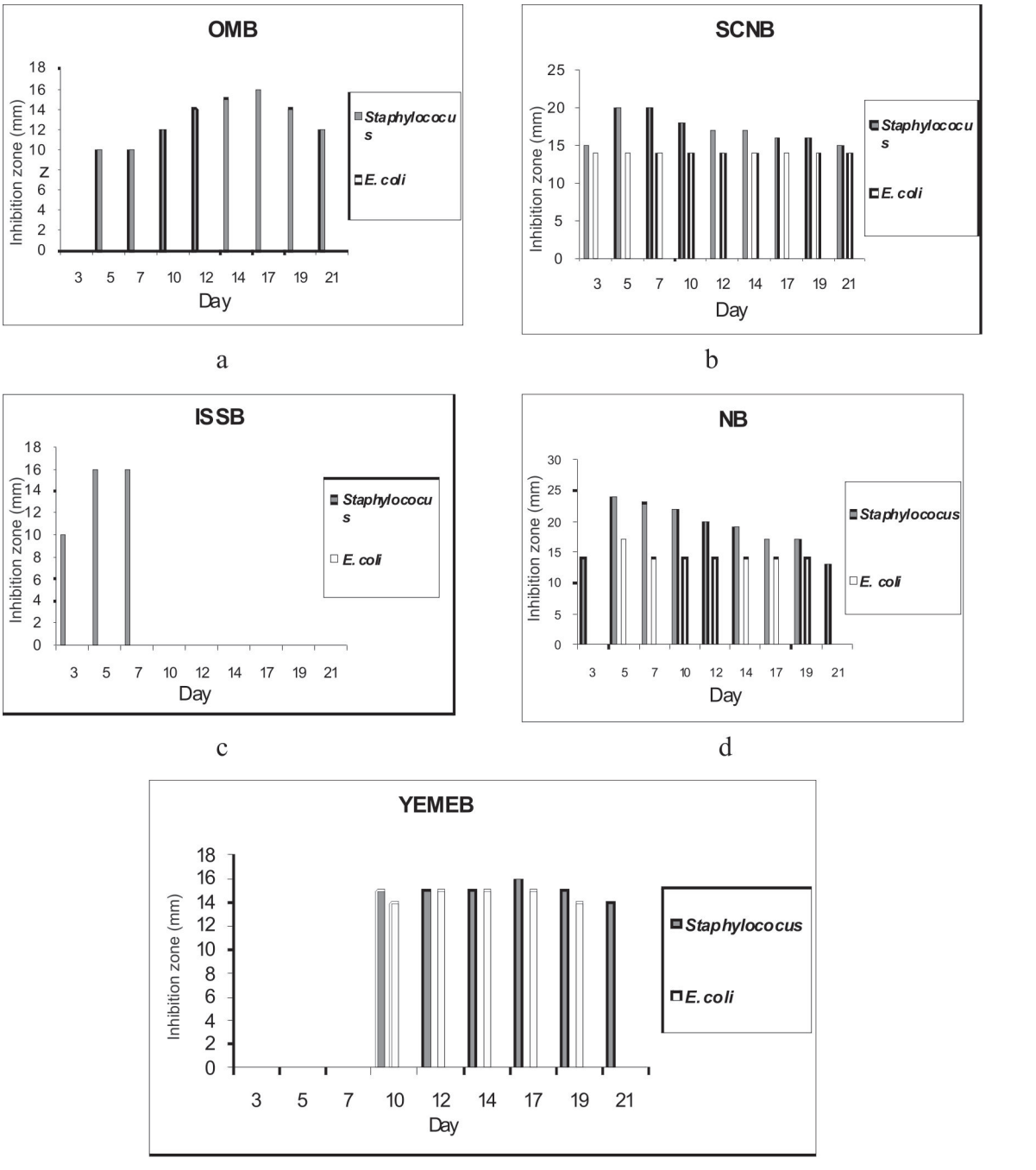


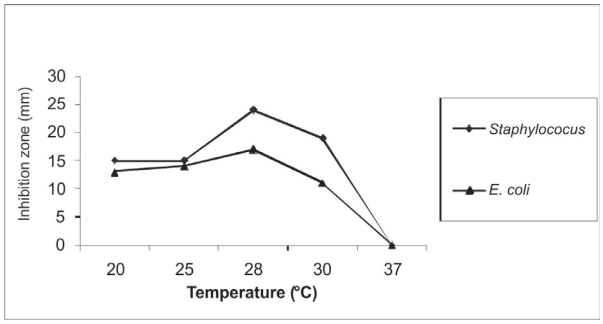
Fig. 1. Production of antibiotic compound by *Streptomyces* N-404 strain in different broth media. a: Oatmeal broth (OB); b: Starch casein nitrate broth (SCNB); c: Inorganic salt starch broth (ISSB); d: Nutrient broth (NB); e: Yeast extract malt extract broth (YEMEB).

study the effect of temperature and pH on the antimicrobial compound production.

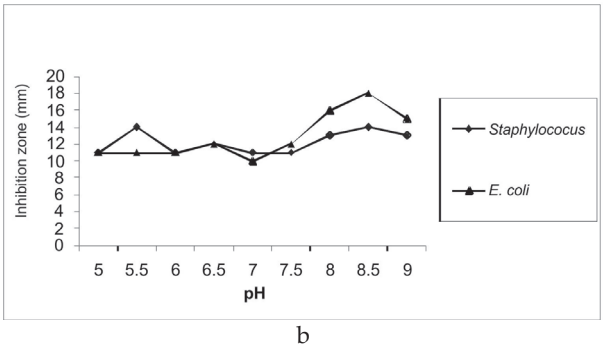
The optimal temperature for antimicrobial production by *Streptomyces* N-404 strain after incubation in NB for 5 days was observed at 28 °C ± 1 with an inhibition zone diameter ranged between 11 and 24 mm. The results reported here are similar to what reported by Ouhdoueh *et al.* (2003) where *Streptomyces* usually produce antibiotics at a temperature near 27 °C. The optimum temperature for *Streptomyces*-producing antibiotics ranges from 26 °C to 30 °C, which are the conditions that were repoted by Egorov (1985) that microorganisms would grow normally and produce antibiotic only if certain optimum temperature of cultivation is ensured. No production of the antimicrobial compound against both tester pathogens was observed at 37 °C ± 1 (Fig. 2a).

The effect of pH on antimicrobial production by *Streptomyces* N404 strain after 5 days of cultivation in NB is shown in (Fig. 2b). Data indicated that the maximum antimicrobial activity occurred at pH 8.5, with an inhibition zone diameter range between 14 and 18 mm. It is clear from the data that the antimicrobial production by N-404 strain at pH 5.5-7.5 was not different from each other as inhibition zones ranged from 10 to 14 mm. However, production was noticed to increase at pH 7.5-8.5, then a slight reduction at pH 9.0 was observed.

The pH of the medium is very important for growth of the microorganism, activity of the enzymes, characteristic of their metabolism, and hence for biosynthesis of metabolites. For this reason, investigators observed that the optimal pH for antibiotic production by *Streptomyces* sp. range between 7.0 and 7.5 (Sirvastava and Singhal 1994; EL-Banna and Winkelmann 1997).



a



b

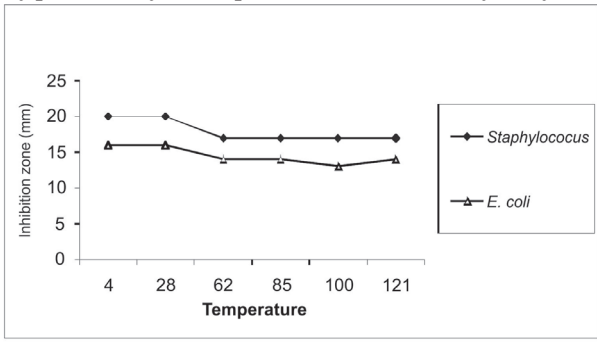
Fig. 2. Effect of temperature (a) and pH (b) on production of antibiotic compound by *Streptomyces* N-404 strain after 5 days of incubation.

Stability of the antimicrobial compound

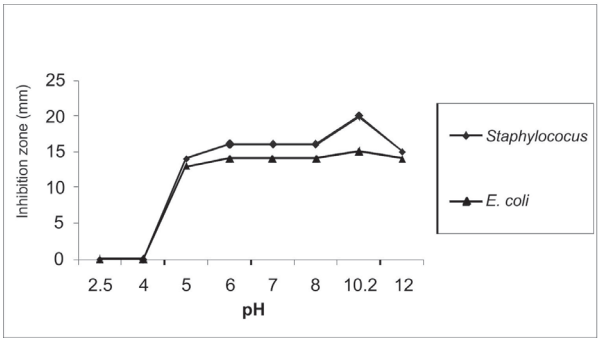
Resistance of the active substance(s) to heat is shown in Fig. 3a. The substance responsible for the inhibition retained activity after heating at temperaturas ranged from 62 to 100 ºC and even after autoclaving with an inhibition zone diameter ranged between 13 and 17 mm (Fig. 3a). The inhibitory substance was found to be stable at a wide range of pH levels (5-12), but no activity was detected at pH 2.5 and 4 (Fig. 3b).

Susceptibility of the antimicrobial compound to different digestive enzymes

Sensitivity of the antimicrobial compound to trypsin, amylase, proteinase-K and lysozyme



a



b

Fig. 3. Stability of the antibiotic compound produced by *Streptomyces* strain N-404 at different (a) temperatures and (b) pH levels

enzymes indicated that they were not completely inactivated by most tested enzymes (Fig. 4). However, this acivity was declined as the antimicrobial compound was treated with proteinase-K.

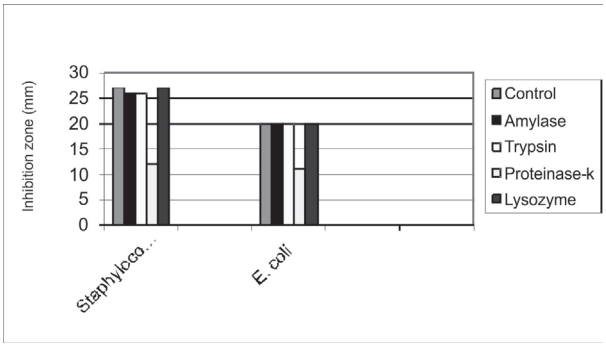


Fig. 4. Inactivation of the antibiotic compound produced by *Streptomyces* N-404 strain by different digestive enzymes and lysozyme.

Comparison of activity of the antimicrobial compound

It is necessary to extract and purify the substance responsible for the antimicrobial activity, so further tests for evaluation can be carried out with a simple ethanolic extraction of the inhibitory compound from cells grown in liquid cultures. When the activity of the concentrated ethanolic extract against the tester pathogenic bacteria was compared to the activity of the broth production medium, results indicated that the activity almost doubled against both tester bacteria (Fig. 5).

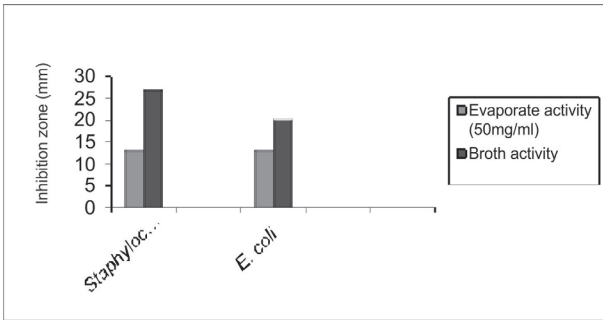


Fig. 5. Activity of broth and the water soluble concentrated ethanolic crude extract against the different test pathogens.

Minimum inhibitory concentration of the antimicrobial compound

Using serial dilution assay starting with a concentration of 10 mg/mL, the minimum inhibitory concentration (MIC) of the water-soluble of the concentrated ethanolic extract was 5 and 10 mg/mL against *S. aureus* and *E. coli*, respectively (Table 1).

Table 1. Minimum inhibitory concentration (MIC) of the water soluble of concentrated ethanolic crude extract of *Streptomyces* N-404 strain.

Pathogen	MIC (mg Concentrated ethanolic crude residues/mL)									
	10	9	8	7	6	5	4	3	2	1
	Inhibition zone diameter (mm)									
Staphylococcus aureus	25	25	25	24	23	21	-	-	-	-
Escherichia coli	12	-	-	-	-	-	-	-	-	-

Table 2. Different cationic (CAT) and anionic (ANA) exchangers and adsorbent (ADS) resins tested for further purification of the active substance(s) after adjusting the broth medium to pH 5 and 9.

Exchanger	Description	Activity against *S. aureus*	
		pH 5	pH 9
Amberlite CG50	CAT, strong		-
Cellulose phosphate	CAT, weak		+
DEAE cellulose	ANA, weak		-
PEI cellulose	ANA, medium		+
Amberlite IRA40	ANA, strong		+
TEAE cellulose	ANA, weak		+
Dowex-2	ANA, strong		+
Hydroxy appetite	ADS	-	-
Aluminum oxide	ADS	-	+
Permutite	ADS	-	-
Zeolite	ADS	-	-

Purification and characterization of the antimicrobial compound from Streptomyces N-404 strain

Results of purification of the concentrated ethanolic extract showed that the active substance in the NB binded to the resins of amberlite CG50, DEAE cellulose, hydroxy appetite, permutite and zeolite (Table 2). Purification experiments for the active compounds out of the permutite column by a gradient solution of 0.05 M KOH revelaled their elution in fractions No. 11 and 12 as one major peak and an activity against *S. aureus* with inhibition zone diameter of 19 mm (Fig. 6). However, this activity was increased to 74% with an inhibition zone diameter of 33 mm after treatment of the eluent with $HCLO_4$ (Fig. 6).

The U.V absorption spectrum of the concentrated ethanolic extract is generally similar to the tested standard antibiotics (gentamycin and kanamycin) which exhibited a maximum absorbance at 200-205 nm (Data not shown). Saadoun and Muhana, 2008 reported an activity of a concentrated water soluble ethanolic extract from culture broth of *Streptomyces* Ds-104 isolate against multi-drug resistant *C. albicans* with a U.V maximum absorbance at 205 nm. Saadoun *et al*. (2009) in their study showed that approximately 50% of reported active screened *Streptomyces* isolates exhibit similar UV-spectra.

Test results indicated that when the active substance was compared to gentamycin (30 mg/mL) and kanamycin (30 mg/mL) in term of their stability under high temperatures (100 °C and autoclaving at 121 °C), basic (pH 12) and acidic (pH 4) conditions, adsorption to charcoal at pH 6 and 9, and TLC pattern, the active substance resemble gentamycin and kanamycin in term of temperature and pH

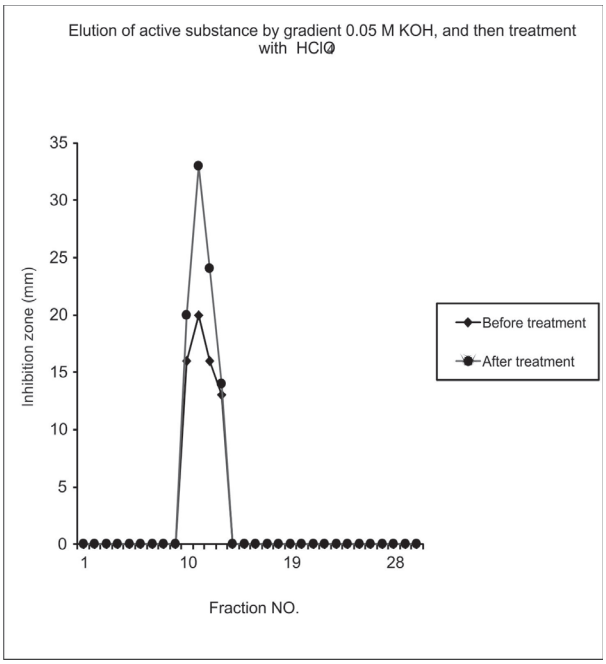


Fig. 6. Purification of the water soluble concentrated ethanolic crude extract through permutite resins and elution of fractions by a gradient solution of KOH and their activity

Table 3. Comparison of the partial purified active substance(s) with gentamicin and kanamycin

Property	Active substance (100 mg/mL)	Gentamicin (30 mg/mL)	Kanamycin (30 mg/mL)
Temp. Stability[a]			
Boiling (100 °C)	Stable	Stable	Stable
Autoclave (121 °C)	Stable	Stable	Stable
pH Stability[a]			
pH 4	Stable	Stable	Stable
pH 12	Stable	Stable	Stable
Adsorption to Charcoal[a]			
pH 9	Weak (24-33 mm)	Not Adsorbed	Not Adsorbed
pH 6	Adsorbed	Not Adsorbed	Not Adsorbed
TLC			
No. of Spots			
R_f value of each spot (cm)	21, 7.5	19.2	19.1
UV Spectrum			
Basic Condition	Data not shown	ND	ND
Acidic Condition	Data not shown	ND	ND

[a]Temperature and pH stability, and adsorption to charcoal under alkaline and acidic conditions were assessed by determining the activity against *S. aureus*.
[b]ND: Not Determined

Fraction /Tube No.	Inhibition Zone Diameter (mm)	
	Before $HCLO_4$ Treatment	After $HCLO_4$ Treatment
1 (10)	16	20 (25%)[a]
2 (11, 12)	19	33 (74%)
3 (13)	16	24 (50%)
4 (14, 15)	12	14 (17%)
Control (Concentrated Evaporate) before elution 17		
Control (Fraction No. 16 + 1M $HCLO_4$)	0	0

[a] Numbers between parentheses represent the percentage increase of inhibition zone diameter

stability (Table 3), but differs from these antibiotics in term of adsorption to charcoal at both pH (Table 3), and UV spectrum. On thin layer chromatography, the active substance from N-404 strain gave 2 spots with R_f value of 1 and 7.5. On the other hand, the R_f values of the test drugs gentamicin and kanamycin were 9.2 and 9.1, respectively.

Results showed that gentamycin (10 mg) and kanamycin (10 mg) were able to inhibit the growth of *Streptomyces* N-404 strain with an average inhibition zone diameter of 25 and 42 mm, respectively. This demonstrates that *Streptomyces* N-404 strain is not producing gentamycin and kanamycin antibiotics.

CONCLUSION

It is very important to continue searching for newer and natural inhibitory bioactive compounds to combat the threat of microbial drug resistance. The unique *Streptomyces* N-404 strain produces possible novel inhibitory bioactive compound other than the standard antibiotics. The activity of *Streptomyces* N-404 strain stressed its potential as antagonists against medical pathogens and as a source of a novel antibiotic.

ACKNOWLEDEMENTS

This research was financed by the Higher Council for Science and Technology/ under the biodiversity of soil microorganisms in Jordan, grant No. 189/00. Appreciation is extended to Jordan University of Science and Technology/Irbid-Jordan and to University of Sharjah/Sharjah-UAE for their administrative support.

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