Biomedicine and Chemical Sciences 1(2) (2022) 35-40

6-BCS-608-75

Contents lists available at:
https://journals.irapa.org/index.php/BCS/issue/view/7

Biomedicine and Chemical Sciences

Journal homepage: https://journals.irapa.org/index.php/BCS

Evaluating the Inhibitory Effect of *Streptomyces* Bacteria against Pathogenic Bacteria and Study its Compatibility with Some Antibiotic Types

Mohsen Hashem Risan[a*], Shams Ahmed Subhi[b], Baydaa Y. Hussain[a]

[a]*College of Biotechnology, Al-Nahrain University, Baghdad – Iraq*
[b]*Al-Turath University College –Iraq*

ARTICLE INFO

Article history:
Received on: November 05, 2021
Revised on: February 22, 2022
Accepted on: February 22, 2022
Published on: April 01, 2022

Keywords:
Streptomyces
Bacteria
Compatibility
Antibiotics

ABSTRACT

All *Streptomyces* sp isolates were screened for their antibacterial activity on Yeast extract-malt extract agar medium (ISP2) using scross-streak technique against two pathogenic bacteria include Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*. Among three *Streptomyces* sp isolates that obtained from Baghdad city (Al-Jadriya), one isolates (B2) didn't show any antibacterial activity against any type of pathogenic bacteria (Gram-negative and Gram-positive bacteria), while two *Streptomyces* sp isolates (B1 and B3) showed antibacterial activity against Gram Two-negative *(Pseudomonas aeruginosa)* and Gram-positive *(Staphylococcus aureus)*. Screening was performed by Agar-Well Diffusion method and growth inhibition zones were measured in millimeters for each of the *Streptomyces* isolates (B1 and B3). Tested isolates have shown potent in *vitro* antibacterial activities against all tested pathogens. The highest activities were shown by isolate B1 against *S. aureus* 19.5 mm, *Pseudomonas aeruginosa* 14 mm. It is also evident that B3 isolate has shown activities against all pathogenic bacteria with inhibition zone diameters ranging between 17 and 13 mm against *S. aureus* and *P. aeruginosa* respectively. The effects of Levofloxacin, Sulfamethoxazole, Ciprofloxacin, Ceftriaxone, Aztreonam, Amikacin and Gentamicin on growth of *Streptomyces* sp were evaluated over a 48 h period. Morphology and growth of *Streptomyces* sp. were not affected by all antibiotics, all *Streptomyces* isolates (B1, B2) were screened for resistance against seven antibiotics, all *Streptomyces* isolates were resistance against all antibiotics.

1. Introduction

Actinomycetes produce about two-thirds of the known antibiotics and among them 80% are made by members of the genus *Streptomyces*, with other genera trailing numerically. Actinomycetes also account for 60% of secondary metabolites with biological activities other than antimicrobial, and again *Streptomyces* species account for 80% of these (Kieser et al., 2000; Amin et al., 2016; Risan et al., 2017; Qasim and Risan 2017; Risan et al., 2017; Al-Rubaye et al., 2018; Risan et al., 2019; Al-Rubaye et al., 2020). *Streptomyces* are Gram positive aerobic bacteria belonging to the phylum Actinobacteria (Stackebrandt et al., 1997). At least 7,000 different secondary metabolites have been discovered in *Streptomyces* isolates (Berdy, 2005). *Streptomyces* synthesize an amazing variety of chemically distinct inhibitors of many different cellular processes. These include antibiotics, fungicides, modulators of the immune response, and effectors of plant growth (Hopwood, 2007). The present work was aimed to evaluating the inhibitory effect of *Streptomyces* bacteria against pathogenic bacteria and study its compatibility with some antibiotic types.

2. Materials and Methods

2.1. Actinomycetes Isolation

About 12 soil samples were gathered from Baghdad city (Al-Jadriya) on December 2020. From the top surface at a depth of 10 cm, soil samples were taken. The samples were placed separately in sterile plastic containers firmly sealed,

[*]**Corresponding author:** Mohsen Hashem Risan, College of Biotechnology, Al-Nahrain University, Baghdad – Iraq
E-mail: **m_risan@yahoo.com**

How to cite:
Risan, M. H., Subhi, S. A., & Hussain, B. Y. (2022). Evaluating the Inhibitory Effect of Streptomyces Bacteria Against Pathogenic Bacteria: Compatibility with Antibiotic Types. Biomedicine and Chemical Sciences, 1(2), 35–40.
DOI: **https://doi.org/10.48112/bcs.v1i2.75**

and transferred to the lab. about three hours, the soil samples were dried in a hot air oven at 60-65°C to reduce the vegetative bacterial population, following that the soil samples with Actinomycetes spores were then sorted into sterile tubes and kept at 4°C until the screening was complete. Actinomycetes were cultured and isolated using a starch-casein-nitrate-agar medium. Before being sterilizing in an autoclave at 121°C for 15 minutes the medium must adjusted to a pH of 7-7.2. and then the medium must allowed to cool around 45-50°C. Before pouring into plates, 50 µg/ml of tetracycline and 50 µg/ml Nystatin, were added. Then the medium was put into the plates in various thicknesses to avoid drying throughout the incubation time (Gesheva et al., 2005).

From dried soil sample only one gram was suspended in 99 ml sterile distilled water. From the stock suspension solution a serial dilutions (10−1 - 10−4) were prepared. Also Transferring 0.1 ml of the spore suspension of dilution and distributing over the surface of agar media with a sterile glass spreader in which were used to culture three petri dishes containing isolation medium. Then, the plates were incubated at 28°C for 7 days. After seven days the plates were examined after the incubation period for typical *Actinomycetes* colonies exhibit cultural characteristics which are circular, tiny, opaque, compact, and commonly colored with white, brown, gray-pink, or other colors. The colonies were examined under an optical microscope to observe their morphology and recognized from fungi colonies. Re-culture the Actinomycetes isolates in nutrient broth and agar slants and store at 4°C for future research and investigation (Gesheva et al., 2005).

2.2. Pathogenic Bacteria Used in The Study

All pathogenic bacteria used in the study were obtained from the College of Biotechnology's Laboratory Microbiology/Mycology. To demonstrate Actinomycetes' antibacterial activity, pathogenic bacteria *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were used as test microorganisms.

2.3. Cultural Characterization of *Streptomyces* Isolates

According to Bergey's Manual of Determinative Bacteriology the isolates were characterized and morphological studies. Under a light microscope, several colonies with diverse morphological and cultural properties, growth, and colour of aerial and substrate mycelia were examined for Gram's straining type, form, and growth of *Streptomyces* isolates. (Vimal et al., 2009; Goodfellow, 1989). The classification of the bacterial population was based on morphological aspects of colonies, such as colony coloration.

2.4. Biochemical Characteristics

Biochemical characterization, various biochemical tests were performed for the identification of the potent isolate *Streptomyces* spp. These tests, including Catalase production, Hydrogen sulfide production, Nitrate reduction, Citrate utilization, Oxidase production, Casein hydrolysis, Indole production, Melanine reaction and Starch (Cowan, 1974; Collins et al., 1995; Deshmukh, 1997).

2.5. Primary Screening for Antibacterial Activity

Primary screening of antagonism was performed on Muller Hinton agar using the perpendicular streak plate method against two test organisms, including *P. aeruginosa* and *S. aureus*. The *Streptomyces* isolates were streaked across the surface of the agar medium at the middle position of the plate and incubated at 30°C for 7 days, in triplicate. After that, the test organisms were streaked perpendicularly with *Streptomyces* growth and the space of 2-3 mm between each two streaks. Then, the plates were incubated at 37°C for 2 days for the test organism growing. After that, the plates were then examined, and the presences of the clear zone between the *Streptomyces* growth and test microorganism indicate growth inhibition of test organisms.

2.6. Effect of Antibiotic Types on the Growth of *Streptomyces* sp.

Effect of antibiotic (Discs) types (Levofloxacin, Sulfamethoxazole, Ciprofloxacin, Ceftriaxone, Aztreonam, Amikacin and Gentamicin) on the growth of *Streptomyces* sp, use Mueller-Hinton medium, antibiotics were inoculated with 0.5 ml of a bacterial suspension containing l07 c.f.u. ml and incubated at 37 °C at 48 h. Diameters (in mm) of growth inhibition zones were measured after incubation at 37°C for 48 h.

3. Results & Discussion

3.1. Isolation of Actinomycetes

Twelve soil samples were collected from Baghdad city (Al-Jadriya) on December 2020. The serial dilution technique was used to isolate actinomycetes from ten different soil sources after inoculating the plates with soil suspension on The starch casein nitrate agar medium supplemented with tetracycline 50 µg/ml and 50 µg/ml Nystatin, the plates were incubated at 28°C for 7 days with a dilution 10-4. The data presented in Table (1) summarize all suspected actinomycetes obtained from the above soil sources on the basis of forming pinpoint colonies with inhibitory or clear zone of inhibition around them as recommended by Oskay et al. (2004). Nystatin reduces fungal growth, whereas tetracycline reduces other bacteria. Colonies size varied, powdery, colour varied from chalky white, buff, brown, pink, red, white, yellow and grey. This was in agreement with that described by Saadoun et al. (2015).

Table 1
Actinomycetes colonies appear on starch casein nitrate agar medium for 7 days

No.	Soil samples sites	Actinomycetes colonies	Total colonies
1	Al-Jadriya	0	
2	Al-Jadriya	1	
3	Al-Jadriya	2	
4	Al-Jadriya	2	
5	Al-Jadriya	3	
6	Al-Jadriya	1	
7	Al-Jadriya	0	
8	Al-Jadriya	3	17
9	Al-Jadriya	0	
10	Al-Jadriya	2	
11	Al-Jadriya	0	
12	Al-Jadriya	3	

The morphology and size of the colonies were about 10 mm in diameter with a relatively smooth surface at the beginning of the growth, white, yellow and grey, it was developed to an aerial mycelium that appeared as granular,

powdery and soft. Stackebrandt et al. (1994) and Ramazani et al. (2013) described actinomycetes colonies being slow growing, glabrous or chalky, aerobic, piled, as well as with different color of aerial and substrate mycelium. In addition, all isolated colonies possess an earthy odour. From 12 soil samples, 17 colonies were obtained. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and colour ranging from white, brown, and grey were selected.

Out of the 17 Actinomycetes colonies. sub-cultured on ISP2 for growth, and incubation of plates for 7 days, only three isolates demonstrated cultural characteristics similar to that of *Streptomyces* sp. three isolates were selected and purified by pure culture techniques of *Streptomyces* sp. All isolates were given a number as B1, B2, and B3 (Table 2). The Growth characteristics of colony on medium ISP2 as (very good) were a prerequisite for isolates selection of *Streptomyces* sp. The results were in agreement with the finding of Zhou et al. (2007).

Table 2
The Growth characteristics of *Streptomyces* colonies on medium ISP2

Medium	Isolates	Growth
ISP2	B1	++++
	B2	++++
	B3	++++
++++: very good		

The results were in agreement with the finding of both Zhou et al. (2007) and Portillo et al. (2009) concerning the isolation process that each plate was often contained one or few colony types ranging from two to four colonies, and from similar habitats the actinomycetes diversity exhibited few different colony types. Kariminik and Baniasadi (2010), mentioned that because of their stringent aerobic metabolisms, actinomycetes.

3.2. Cultural and Morphology Characteristics of *Streptomyces* sp

The all *Streptomyces* sp isolates were Gram's stain (Table 3). Young cultures (5-7 days old) produced Substrate mycelia, Branched or Fragments. The colours of the substrate mycelia and aerial mycelia of the isolates, varied from colourless to white, brown, and grey on ISP 2 (Table 3).

Table 3
Cultural and morphology characteristics of *Streptomyces* isolates after 7 days growth on ISP 2 medium

No.	Characteristic	*Streptomyces* isolates		
		B1	*B2*	*B3*
1	Gram's stain	+	+	+
2	Substrate mycelia	Fragments	Fragments	Fragments
3	Colour of aerial mycelia	white	White - Grey	White- orange
4	Colour of substrate mycelia	white – brown	Grey	brown
5	Colour of soluble pigment	grey-violet	grey	grey

3.3. Biochemical Characteristics

The biochemical properties are summarized in (Table 4). All of the isolates belonging to the *Streptomyces* sp.

Table 4
Biochemical characteristics of *Streptomyces* sp isolates after 7 days growth on ISP 2 medium

No.	Characteristic	B1	B2	B3
1	Catalase production	+	+	+
2	Hydrogen sulfide production	-	-	-
3	Nitrate reduction	+	+	+
4	Citrate utilization	-	-	-
5	Oxidase production	-	-	-
6	Casein hydrolysis	+	+	+
7	Indole production	-	-	-
8	Melanine reaction	-	-	-
9	Starch	+	+	+

3.4. Screening for *Streptomyces* sp isolates activity

All *Streptomyces* sp isolates (B1, B2 and B3) were screened for their antibacterial activity on Yeast extract-malt extract agar medium (ISP2) using scross-streak technique against two pathogenic bacteria including Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*). Among three *Streptomyces* sp isolates that obtained from Baghdad city (Al-Jadriya), one isolates (B2) didn't show any antibacterial activity against any type of pathogenic bacteria (Gram-negative and Gram-positive bacteria), while two *Streptomyces* sp isolates (B1 and B3) showed antibacterial activity against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) (Table 5).

Table 5
Primary screening of *Streptomyces* isolates using scross-streak technique on Yeast extract-malt extract agar medium

Isolates	Gram-positive	Gram-negative	Note
	S. aureus	*P. aeruginosa*	
B1	+	+	Selected
B2	-	-	Neglected
B3	+	+	Selected

Screening was performed by Agar-Well Diffusion method and growth inhibition zones were measured in millimeters for each of the *Streptomyces* isolates (B1 and B3), the results are shown in Table (6). Tested isolates have shown potent *in vitro* antibacterial activities against all tested pathogens. The highest activities were shown by isolate B1 against *S. aureus* 19.5 mm, *Pseudomonas aeruginosa* 14 mm. It is also evident that B3 isolate has shown activities against all pathogenic bacteria with inhibition zone diameters ranging between 17 and 13 mm against *S. aureus* and *Pseudomonas aeruginosa* respectively.

Table 6
Inhibition zones (mm) by different *Streptomyces* isolates against pathogenic bacteria

Streptomyces Isolates	Zone of inhibition (mm)	
	S. aureus	*P. aeruginosa*
B1	19.5	14
B2	17	13

Study Pandey et al. (2004) Antibacterial activity of actinomycetes. A total of 106 actinomycetes were subjected to primary screening by perpendicular streak method

against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Enterobacter aerogens, Escherichia coli, Klebsiella* species, *Proteus* species, *Pseudomonas* species, *Salmonell typhi* and *Shigella* species) test bacteria. It was observed that 2 isolates were active against only Gram-negative bacteria, 8 against Gram-positive and 26 against both Gram-positive and Gram-negative bacteria, 36 isolates were subjected to secondary screening by agar well method. Selected isolates (20) from the secondary screening belonged to the genera *Streptomyces* (10 isolates). Finally, one isolate (*Streptomyces* species) was selected. The antibacterial substances were extracted with ethyl acetate from isolate inoculated starch-casein broth fermented for 7 days at 28°C by solvent extraction method. Minimum bactericidal concentration (MBC) of ethyl acetate extract against *Staphylococcus aureus* were 5 mg/ml for *Streptomyces* species. Thin layer chromatography (TLC) of the ethyl acetate extracts were carried out in duplicate using Chloroform: methanol (4:1) as solvent system and Tetracycline as reference antibiotic. Under UV light they gave greenish yellow spots with Rf value 0.88 for the antimicrobial from *Streptomyces* species. In bioautography (using *Staphylococcus aureus* as test organism) inhibition zones were obtained and they were associated with the yellowish green spots of the chromatogram as detected under UV light.

3.5. Effect of antibiotic types on the growth of *Streptomyces* sp

The results effect of antibiotic types (Levofloxacin, Sulfamethoxazole, Ciprofloxacin, Ceftriaxone, Aztreonam, Amikacin and Gentamicin) on the growth of *Streptomyces* sp show in table (7) and fig (1). The effects of Levofloxacin, Sulfamethoxazole, Ciprofloxacin, Ceftriaxone, Aztreonam, Amikacin and Gentamicin on growth of *Streptomyces* sp were evaluated over a 48h period. Morphology and growth of *Streptomyces* sp were not affected by all antibiotics. All *Streptomyces* isolates (B1, B2) were screened for resistance against seven antibiotics, all *Streptomyces* isolates were resistance against all antibiotics.

Table 7
Effect of antibiotic types on the growth of *Streptomyces* sp isolates

No.	Antibiotic	Number of isolates	zones of inhibition(mm)
1	Levofloxacin	N=2	0
2	Sulfamethoxazole	N=2	0
3	Ciprofloxacin	N=2	0
4	Ceftriaxone	N=2	0
5	Aztreonam	N=2	0
6	Amikacin	N=2	0
7	Gentamicin	N=2	0

Fig. 1. Effect of antibiotic types on the growth of *Streptomyces* sp isolates

Antibiotic inhibitory capabilities of *Streptomyces* populations differed between locales. Competitive 'hot spots' that support *Streptomyces* populations that are effective inhibitors of resource rivals could be competitive 'hot spots' that have selected for *Streptomyces* populations that are broad and very potent inhibitory phenotypes. Resource competition may be less relevant to *Streptomyces* fitness in areas where populations have little inhibitory capacity, or populations may be niche varied (Kinkel et al., 2014).

Streptomyces communities with limited inhibitory capacity, on the other hand, may need special conditions to create antibiotics or produce antibiotics that inhibit species other than the indicator overlays used in this investigation. The findings also showed there is a positive relationship between inhibitory capabilities among *Streptomyces* populations and niche overlap from various areas supports the theory that resource competition pushes local *Streptomyces* populations to select for antibiotic inhibitory phenotypes. There are other mediating competition strategies used, such as signaling or strong selection caused of factors instead of resource competition like predation, abiotic stress, and parasitism (Yim et al., 2007; Vaz Jauri et al., 2013; Weekers et al., 1993; Ashelford et al., 2003).

The relationship between antibiotic inhibition growth and niche overlap could be complex, depending on the region due to the different mechanisms of competition and subsequent selection (Thompson, 2005; Kinkel et al., 2014). On a global scale, the enormous diversity of *Streptomyces* antibiotic phenotypes may be attributable to resource competition (Czárán et al., 2002). In agreement with past findings that the *Streptomyces* appeared resistant to the antibiotics (wide spectrum) that used in clinical practice (Wiener et al., 1998; Kinkel et al., 2014). As a results showed that *Streptomyces* resistance to wide spectrum antibiotics of various locations could be effect on the manufacturing of certain antibiotics. Antibiotic resistance could be convinced of efflux pumps activation of broad-spectrum or by inducing resistance mutations (Martinez et al., 2009).

Antibiotic resistance dynamics in *Streptomyces* populations diverge from resource rivalry dynamics, as evidenced by the absence of connection between niche overlap and capability of antibiotic resistance across areas. *Streptomyces* from OTU1 that originated in MN1 were more resistant to rifampicin and streptomycin than those from other sites. (Ant and PanFS), suggesting there are different selective pressures for resistance in each of these regions. Adaptive radiation may help generate large variability in antibiotic resistance phenotypes by allowing species from the same phylogenetic groupings to adapt to different selection pressures across the landscape (Wiener et al., 1998).

4. Conclusions

Morphology and growth of *Streptomyces* sp, were not affected by Antibiotics Levofloxacin, Sulfamethoxazole, Ciprofloxacin, Ceftriaxone, Aztreonam, Amikacin and Gentamicin antibiotics.

Competing Interests

The authors have declared that no competing interests exist.

References

Al-rubaye, T. S., Risan, M. H., & Al-Rubaye, D. (2020). Gas chromatography-mass-spectroscopy analysis of bioactive compounds from Streptomyces spp. isolated from Tigris river sediments in Baghdad city. Journal of Biotechnology Research Center, 14(1), 63-71. https://doi.org/10.24126/jobrc.2020.14.1.590

Al-Rubaye, T. S., Risan, M. H., Al-Rubaye, D., & Radi, R. O. (2018). Characterization of marine Streptomyces spp. bacterial isolates from Tigris river sediments in Baghdad city with Lc-ms and 1 HNMR. Journal of Pharmacognosy and Phytochemistry, 7(5), 2053-2060.

Amin, S. M., Rasin, M. H., & Abdulmohimin, N. (2016). Antimicrobial and Antioxidant Activities of Biologically Active Extract from Locally Isolated Actinomycetes in Garmian Area. Journal of Garmian University, 1(10). 625-639.

Ashelford, K. E., Day, M. J., & Fry, J. C. (2003). Elevated abundance of bacteriophage infecting bacteria in soil. Applied and environmental microbiology, 69(1), 285-289. https://doi.org/10.1128/AEM.69.1.285-289.2003

Berdy, J. (2005). Bioactive microbial metabolites. The Journal of antibiotics, 58(1), 1-26. https://doi.org/10.1038/ja.2005.1

Collins, C. H.; Lyne, P. M. and Grange J. M. (1995). Microbiological methods. 7th edition, Butterworth Heinemann Ltd. London.

Cowan, S. T. (1974). Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd Edn. London: Cambridge University Press.

Czárán, T. L., Hoekstra, R. F., & Pagie, L. (2002). Chemical warfare between microbes promotes biodiversity. Proceedings of the National Academy of Sciences, 99(2), 786-790. https://doi.org/10.1073/pnas.012399899

Deshmukh, A. (1997): Hand book of media, stains and reagents in microbiology. PAMA publication. 14.

Gesheva, V., Ivanova, V., & Gesheva, R. (2005). Effects of nutrients on the production of AK-111-81 macrolide antibiotic by Streptomyces hygroscopicus. Microbiological research, 160(3), 243-248. https://doi.org/10.1016/j.micres.2004.06.005

Goodfellow et al., 1988. Actinomycetes in Biotechnology. Academic Press, London.

Hopwood D. A. (2007). Actinomycetes and Antibiotics; Antibiotic Discovery and Resistance. Streptomyces in Nature and Medicine: The Antibiotic Makers. Oxford University Press, New York, NY. pp. 8-50.

Kariminik, A., & Baniasadi, F. (2010). Pageantagonistic activity of Actinomycetes on some Gram negative and Gram positive bacteria. World Applied Sciences Journal, 8(7), 828-832.

Kieser et al., 2000. General introduction to actinomycete biology. Practical Streptomyces Genetics (2nd ed.). John Innes Foundation, Norwich, England. pp. 2-41.

Kinkel, L. L., Schlatter, D. C., Xiao, K., & Baines, A. D. (2014). Sympatric inhibition and niche differentiation suggest alternative coevolutionary trajectories among Streptomycetes. The ISME journal, 8(2), 249-256. https://doi.org/10.1038/ismej.2013.175

Martinez, J. L., Sánchez, M. B., Martínez-Solano, L., Hernandez, A., Garmendia, L., Fajardo, A., & Alvarez-Ortega, C. (2009). Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. FEMS microbiology reviews, 33(2), 430-449. https://doi.org/10.1111/j.1574-6976.2008.00157.x

Oskay, A. M., Üsame, T., & Cem, A. (2004). Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African journal of Biotechnology, 3(9), 441-446. https://doi.org/10.5897/AJB2004.000-2087

Pandey, B., Ghimire, P., & Agrawal, V. P. (2004). Studies on the antibacterial activity of the Actinomycetes

isolated from the Khumbu Region of Nepal. Journal Biology Science, 23, 44-53.

Portillo, M. C., Saiz-Jimenez, C., & Gonzalez, J. M. (2009). Molecular characterization of total and metabolically active bacterial communities of "white colonizations" in the Altamira Cave, Spain. Research in microbiology, 160(1), 41-47. https://doi.org/10.1016/j.resmic.2008.10.002

Qasim, B., & Risan, M. H. (2017). Anti-tumor and Antimicrobial Activity of Antibiotic Produced by Streptomyces spp. World Journal of Pharmaceutical Research, 6(4), 116-128.

Ramazani, A., Moradi, S., Sorouri, R., Javani, S., & Garshasbi, M. (2013). Screening for antibacterial activity of Streptomyces species isolated from Zanjan province, Iran. Int J Pharm Chem Biol Sci, 3(2), 342-349.

Risan, M. H., Jafar, R. A., & Subhi, S. A. (2019). Isolation, characterization and antibacterial activity of a Rare Actinomycete: Saccharopolyspora sp. In Iraq. East African Scholars Journal of Biotechnology and Genetics, 1(4), 60-49.

Risan, M. H., Qasim, B., Abdel-jabbar, B., & Muhsin, A. H. (2017). Identification Active Compounds of Bacteria Streptomyces Using High-Performance Liquid Chromatography. World Journal of Pharmaceutical and Life Sciences, 3(6), 91-97.

Saadoun, I., Al-Joubori, B., & Al-Khoury, R. (2015). Testing of production of inhibitory bioactive compounds by soil Streptomycetes as preliminary screening programs in UAE for anti-cancer and anti-bacterial drugs. Int. J. Curr. Microbiol. App. Sci, 4(3), 446-459.

Stackebrandt, E., & GOEBEL, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. International journal of systematic and evolutionary microbiology, 44(4), 846-849.

Stackebrandt, E., Rainey, F. A., & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, Actinobacteria classis nov. International Journal of Systematic and Evolutionary Microbiology, 47(2), 479-491. https://doi.org/10.1099/00207713-47-2-479

Thompson J. N. (2005) The Geographic Mosaic Of Coevolution. University of Chicago Press, Chicago.

Vaz Jauri, P., Bakker, M. G., Salomon, C. E., & Kinkel, L. L. (2013). Subinhibitory antibiotic concentrations mediate nutrient use and competition among soil Streptomyces. PLoS One, 8(12), e81064. https://doi.org/10.1371/journal.pone.0081064

Vimal, V., Rajan, B. M., & Kannabiran, K. (2009). Antimicrobial activity of marine actinomycete, Nocardiopsis sp. VITSVK 5 (FJ973467). Asian J Med Sci, 1(2), 57-63.

Weekers, P. H., Bodelier, P. L., Wijen, J. P., & Vogels, G. D. (1993). Effects of grazing by the free-living soil amoebae Acanthamoeba castellanii, Acanthamoeba polyphaga, and Hartmannella vermiformis on various bacteria. Applied and Environmental Microbiology, 59(7), 2317-2319. https://doi.org/10.1128/aem.59.7.2317-2319.1993

Wiener, P., Egan, S., & Wellington, E. M. H. (1998). Evidence for transfer of antibiotic-resistance genes in soil populations of streptomycetes. Molecular ecology, 7(9), 1205-1216. https://doi.org/10.1046/j.1365-294x.1998.00450.x

Yim, G., Huimi Wang, H., & Davies Frs, J. (2007). Antibiotics as signalling molecules. Philosophical Transactions of the Royal Society B: Biological Sciences, 362(1483), 1195-1200. https://doi.org/10.1098/rstb.2007.2044

Zhou, J. P., Gu, Y. Q., Zou, C. S., & Mo, M. H. (2007). Phylogenetic diversity of bacteria in an earth-cave in Guizhou Province, Southwest of China. Journal of microbiology, 45(2), 105-112.