World Journal of
Experimental Biosciences

Volume 12, Number 1: 21 - 25
ISSN: 2313-3937
2024

Research article

Antagonistic Interactions Between Soil-Isolated Actinomycetes and Pathogenic Bacterial Strains

Ayat Abdalmutalib Mahdi[1], Sajjad Falah Hassan[1], Rawan abdulkadir zaki[1], Hiba Tawfiq Ali[1], Jenan Atiyah Ghafil[1], Ayaid Khadem Zgair[1]*,

ABSTRACT

Bacterial resistance to commonly used antibiotics for treating infections is the biggest challenge facing clinicians. Previous research on antibiotic-producing actinomycetes has been limited. This study aims to explore the potential of identifying actinomycete isolates that may produce antibiotics more effective than current treatments for bacterial infections. Fifty soil samples were collected from various regions in central Iraq to isolate actinomycetes. Ten clinical bacterial isolates of Staphylococcus aureus, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Enterococcus faecalis*, which were resistant to multiple antibiotics, were obtained and maintained for use in experiments. The double-layer of agar and spot assay was used to detect antagonistic interactions between actinomycete and pathogenic bacterial isolates (ten isolates). The Kirby-Bauer method was employed to evaluate the response of these isolates to four different antibiotics (ofloxacin (OF), gentamicin (CN), amoxicillin (AX), vancomycin (VA). The experiments demonstrated successful isolation of actinomycetes and validated the effectiveness of the employed method. The results indicated that the clinical isolates were highly resistant to the antibiotics tested. Nine out of ten isolates showed antagonistic reactions to actinomycete bacteria. This suggests that soil-derived actinomycetes can be isolated and possess strong antagonistic activity against bacteria resistant to various antibiotics. Such isolates could potentially be used to develop more effective antibiotics than those tested in this study.

Keywords: Actinomycetes, Antagonism, Antibiotics, Clinical pathogenic bacteria.

Citation: Mahdi AA, Hassan SF, Zaki RA, Ali HT, Ghafil JA, Zgair AK. (2024) Antagonistic Interactions Between Soil-Isolated Actinomycetes and Pathogenic Bacterial Strains. *World J Exp Biosci* **12**: 20-25.

Received May 12, 2024; Revised June 18, 2024; Accepted: June 24, 2024

1. INTRODUCTION

The wide distribution of multidrug-resistant bacteria poses a grave threat to global health by overwhelming our current antibiotic arsenal and pushing infection-associated morbidity and mortality to new heights [1]. Pathogens, including Staphylococcus aureus, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Enterococcus faecalis*, are rapidly acquiring resistance to entire families of drugs, underscoring the pressing need for new, effective treatment options [2].

The World Health Organization has repeatedly called for the identification of new bioactive molecules, with a particular emphasis on those sourced from nature, to outpace these resistant strains [3]. Within this research, soil-dwelling microorganisms, especially actinomycetes, are attracting intense interest due to their established ability to produce a wide array of complex and biologically potent secondary metabolites, which serve as antimicrobial agents [4].

* Correspondence: **Ayaid Khadem Zgair**. E-mail: ayaid.zgair@sc.uobaghdad.edu.iq
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
Full list of author information is available at the end of the article.

Actinomycetes are Gram-positive filamentous bacteria that belong to the genus Streptomyces, which produces a wide variety of antibiotics [5], antifungals, antivirals, immunosuppressive agents, and antitumor compounds [6]. It is predictable that more than two-thirds of naturally derived antibiotics, including streptomycin, tetracyclines, erythromycin, and rifamycin. Their notable metabolic diversity stems from the presence of big metabolic organizing gene groups that encode polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs) that enable the formation of secondary metabolites with exclusive mechanisms of accomplishment [7]. Despite extensive studies on these types of bacteria, only a few actinomycete species have been identified, suggesting that many undiscovered strains in the environment may serve as reservoirs for novel bioactive compounds [8].

Soil is one of the most complex and nutrient-rich environments in actinomycete habitats. To support growth and metabolite production, optimal environmental conditions are created. The chemical and physical properties of soil, including nutrient content, pH, and organic matter, are believed to influence the diversity and biosynthetic potential of these microorganisms [9]. Soil actinomycetes play a vital ecological role by decomposing organic matter and inhibiting the growth of other microorganisms through the production of antimicrobial metabolites. This natural antagonistic behavior makes soil-dwelling actinomycetes attractive sources for screening against pathogenic bacteria, especially multidrug-resistant (MDR) strains [10].

Recently, the potential of soil actinomycetes in screening for new antibiotic agents has gained interest. For example, antibiotic-active actinomycete isolates collected from agricultural soils, forest soils, and extreme environments (such as deserts and saline soils) have shown inhibitory activity against various clinical pathogens. In many cases, actinomycetes exhibit a vast and largely unexplored diversity across different geographic locations and soil types. Due to the limited understanding of the diversity and distribution of actinomycetes, there is still much to be discovered and understood about them. The reason for studying soil actinomycetes partly stems from their potential to contain unique strains that produce new antimicrobials. Characterizing these strains for their inhibitory activity and antagonism against clinically significant bacteria is an essential part of early-stage screening in the search for potential antibiotics [11]. This finding can have a significant impact in the war against MDR bacteria, which are responsible for high-risk infectious bacterial diseases. This type of research may provide a novel approach to antimicrobial research areas.

The current study aims to investigate the antagonistic interactions between soil-isolated actinomycetes and selected pathogenic bacterial strains. To achieve the study's goal, actinomycetes were isolated from various soil samples and evaluated for their antibacterial activities using standard in vitro assays.

2. MATERIALS and METHODS

2.1. Soil sample collection

The previous standard method, as described by Khudhair et al. (2023), was followed to collect soil samples from various environmental areas in central Iraq [5]. In this experiment, clean and sterile universal glass containers were used. The sampling sites were selected to represent diverse environments (different land uses, vegetation types, moisture levels, and desert areas) to determine the highest probability of isolating actinomycete species. Fifty soil samples were collected from various sites in Baghdad city and its surrounding areas.

The soil samples were collected from the sub-top layer (typically the top 6-12 cm) to get the highest active soil microbial community. The clean and sterilized device was used to collect the soil sample. The surface debris and organic matter have to be avoided. Multiple samples were collected across the area of interest and mixed to create a composite sample for representative analysis. The information labels were put onto the containers. The location, date, depth, and any other relevant information were included in the labels of the containers. The samples were kept in a refrigerator until processing [11].

2.2. Clinical bacterial isolates

In the current study, clinical isolates of *S. aureus, E. coli, E. faecalis, P. aeruginosa*, and *K. pneumoniae* were obtained from the Postgraduate Laboratory at the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. The clinical isolates were maintained by inoculating them onto nutrient broth and nutrient agar, and then incubating for 18 h at 37°C. They were subsequently stored at 4°C for several weeks. For long-term storage, the bacterial isolates were stored in nutrient broth containing 20 % glycerol at –20°C [11].

2.3. Kirby-Bauer method

The standard method of Yao et al. (2021) was followed to perform the Kirby–Bauer disk diffusion test to identify the susceptibility patterns of ten clinical isolates, including *S. aureus, E. coli, E. faecalis, P. aeruginosa*, and *K. pneumoniae* to the different antibiotics [ofloxacin (of), gentamycin (CN), amoxicillin (AX), vancomycin (VA)]. The diameters were compared with the measured diameters of the Clinical and Laboratory Standards Institute (CLSI) breakpoint charts to determine the Sensitivity (S), Intermediate (I), and Resistance (R) of the bacteria to the above antibiotics [12].

2.3. Isolation and identification of actinomycetes

One gram of soil samples was added to 10 ml of normal saline. The soil samples were double-fold diluted with normal saline. Hundred microliters of each dilution were cultured on the nutrient agar. The plates were incubated for 5-7 days at 37 ºC. The previous method of Gurung et al. (2010) was followed to isolate and identify the isolated actinomycetes. The morphological characteristics of colonies and bacterial filaments, as observed under a light microscope after post-staining with the Gram stain, were used to identify the genus of the isolated actinomycetes [13]. The pure culture of isolated Actinomyces was stored for short-term use by streaking on nutrient agar and then stored at 4°C for two weeks. In the long term, the bacteria were stored at -20 ºC in nutrient broth containing 20 % glycerol.

2.4. Antagonism effect

The agar double layer and spot assay were used to check the antagonistic effect of isolated Actinomycetes and pathogenic bacteria. Briefly, the nutrient plate was divided into several squares using the scale. The first nutrient layer was poured into the plate. After solidification in each square, the spots of actinomyces were done. The plates were incubated at 37 ºC for 2-4 days. The second layer of nutrient agar (40 ºC) was poured. After solidification, the streaking of pathogenic bacteria was done on each plate. The plates were incubated at 37°C for 18 hours. The clear zone appears at each spot, indicating the antagonistic effect between the actinomycetes and pathogenic bacteria. Figure 1 illustrates the procedure's details.

2.5. Statistical analysis

The results were expressed as the mean ± standard deviation (SD).

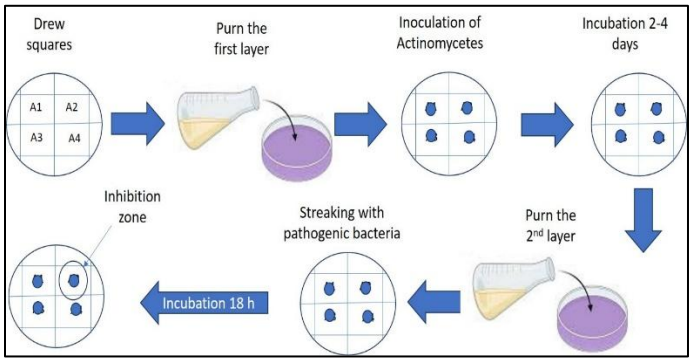

Fig. 1. The diagram of the procedure of the agar double layer and spot assay was used to check the antagonistic effect of isolated Actinomycetes and pathogenic bacteria.

3. RESULTS

3.1. Actinomycete isolate

From five soil samples, we successfully isolated one Actinomycete. This isolate was suspected, based on its morphological characteristics, to be Saccharomyces. We do not have the full facilities to identify the species of the bacteria. The most important issue that we focused on is the antagonism of this isolate with the pathogenic bacterial isolates. The number of isolates used in the current study was ten isolates.

3.2. Kirby-Bauer method

The present study showed the number of clinical isolates that responded to different antibiotics (ofloxacin (OF), gentamycin (CN), amoxicillin (AX), vancomycin (VA). The results indicated that the number of isolates resistant to the four antibiotics was higher than the number of isolates sensitive to them. The study proved that the isolates used in the current study exhibited multidrug resistance.

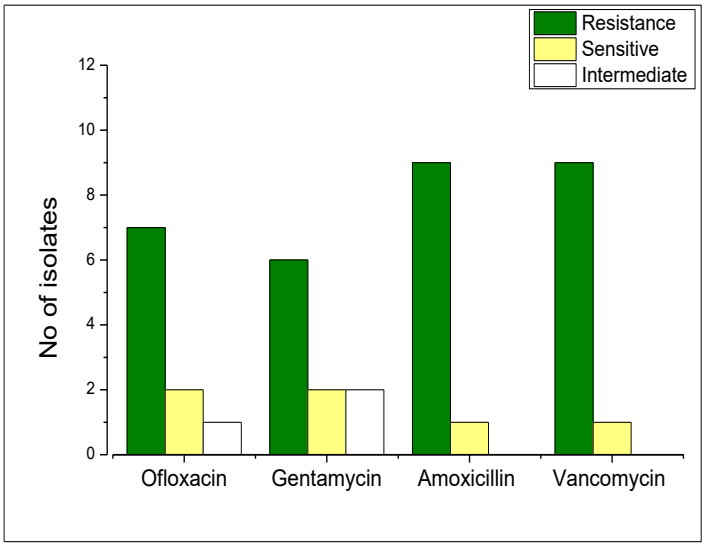

Fig. 2. The susceptibility pattern of ten clinical bacterial isolates (*S. aureus, E. coli, E. faecalis, P. aeruginosa,* and *K. pneumoniae*) to various antibiotics (ofloxacin (OF), gentamicin (CN), amoxicillin (AX), vancomycin (VA).

3.3. Antagonistic effect of actinomycete

The present study demonstrated that the actinomycete isolates exhibited an antagonistic effect against nine isolates of ten different clinical bacterial strains. The only isolate (P. aeruginosa) does not show an effect due to the antagonistic effect of actinomycetes. The isolates were multidrug-resistant; thus, the actinomycetes may produce a higher antibacterial effect than the antibiotics used in the current study. The present study is a prospective study in the fight against multidrug-resistant bacteria.

Table 1. The antagonism of actinomycete isolates and ten clinical bacterial isolates. Positive indicates the inhibitory zone, while the negative indicates no inhibitory response.

No	Isolates	Response to Actinomycetes
1.	*S. aureus*	Positive
2.	*S. aureus*	Positive
3.	*E. coli*	Positive
4.	*E. coli*	Positive
5.	*E. faecalis*	Positive
6.	*E. faecalis*	Positive
7.	*P. aeruginosa*	Negative
8.	*P. aeruginosa*	Positive
9.	*K. pneumoniae*	Positive
10.	*K. pneumoniae*	Positive

4. DISCUSSION

The emergence and widespread outbreak of infection with antibiotic-resistant bacterial pathogens are one of the biggest challenges for healthcare systems worldwide [14]. The present research investigates the potential of soil actinomycetes as leads for the development of novel antimicrobial compounds, particularly against multidrug-resistant (MDR) bacteria. The tests revealed that nine out of ten clinical isolates the tests were conducted on, that is, S. aureus, E. coli, K. pneumoniae, P. aeruginosa, and E. faecalis, were extremely sensitive to actinomycete isolates despite them having been found to be resistant to conventional antibiotics such as ofloxacin, gentamicin, amoxicillin, and vancomycin. The observation aligns with earlier studies that have suggested the potential of actinomycetes, particularly those of the Streptomyces family, to accumulate secondary metabolites with high antibacterial activity [15].

The extremely high rate of antibiotic resistance among the clinical isolates in this study concords with other findings emerging from other regions of the globe, where, for instance, resistance to classes of drugs such as broad-spectrum antibiotics has been rising at an alarming rate [16]. For instance, *P. aeruginosa* and *K. pneumoniae* are both multidrug efflux pumps and β-lactam enzymatic breakdown [17], while *E. faecalis* is commonly said to harbor vancomycin resistance genes [18]. The poor efficacy of the tested drugs in this study highlights the need for alternative strategies, including the exploration of natural sources for novel antimicrobial drugs.

The application of double-layer agar and spot assay was an effective initial technique for screening antagonistic interactions. The large proportion of positive antagonistic reactions (90%) suggests that the central Iraqi soil-derived actinomycetes contain diverse bioactive compounds with activity against pathogenic bacteria. That one of the clinical isolates failed to exhibit antagonistic interaction could be attributed to either inherent resistance factor(s) or failure to produce specific secondary metabolites active against the strain.

Soil remains an underexploited reservoir of new actinomycete species, especially in regions with unique environmental conditions [19]. Iraqi soil, with its dense ecological niches, can be an excellent source of novel strains with unidentified biosynthetic pathways. The isolation of such highly antagonistically active

actinomycetes in the current work again strengthens the notion that regional soil microbial diversity could be exploited for antibiotic drug discovery [20]. Previous research has shown that new actinomycete isolates from understudied environments tend to yield structurally novel, highly active antimicrobial metabolites, indicating a high likelihood of discovering new antibiotics with clinical significance [21].

The results of the present research provide impetus for further elucidation of the active metabolites produced by these isolates. These include purification, structural studies, and identification of the mode of action of these compounds. Additional genomic and metagenomic strategies can be employed to identify biosynthetic gene clusters for antibiotic formation, which could lead to the discovery of totally new types of antibiotics. This study validates the potential of soil-isolated actinomycetes as a source of novel antimicrobial compounds to manage MDR pathogens. The high-level antagonistic activity suggested that these isolates may be a source of clinically effective antibiotics, meeting the growing issue of antibiotic resistance.

5. Conclusion

The discovery of a new antibiotic could significantly benefit public health by enhancing the fight against antibiotic-resistant bacteria. The current study showed the potential to isolate Actinomycetes bacteria from soil in central Iraq. This isolate exhibited antagonistic activity against various clinically resistant bacteria (*S. aureus, E. coli, E. faecalis, P. aeruginosa*, and *K. pneumoniae*) to four main antibiotics (ofloxacin (OF), gentamicin (CN), amoxicillin (AX), vancomycin (VA)) that are routinely used in treating bacterial infections. This research is a step toward developing a new antibiotic derived from these bacteria (Actinomycetes), which could help limit the spread of infectious bacterial diseases and improve public health.

Acknowledgments

We would like to thank the staff members of the soil laboratory at the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. We would also like to thank the clinical lab at the Department of Biology, College of Science, University of Baghdad, for their valuable assistance in isolation and identification of the clinical isolates. Moreover, special thanks are due to all the hospitals in Baghdad, which helped us confirm the clinical isolates.

Funding information

This work received no specific grant from any funding agency.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

The study was carried out after receiving ethical approval from the ethics committee of the Ministry of Health, Iraq, with serial number MoH/1234/00448, 22nd December, 2023

Author contributions

Mahdi AA: Resources; Methodology.
Zaki RA: Methodology
Ali HT: Methodology
Hassan SF: Methodology
Jenan A. Ghafil: Resources; Supervision; Validation; Roles/Writing, Writing – review.
Ayaid K. Zgair: Investigation; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; and Writing - review & editing.

REFERENCES

[1] **Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N,** et al. (2023) Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare (Basel)* **11**(13):1946. doi: 10.3390/healthcare11131946. PMID: 37444780; PMCID: PMC10340576.

[2] **Rostkowska OM, Kuthan R, Burban A, Salińska J, Ciebiera M,** et al. (2020) Analysis of Susceptibility to Selected Antibiotics in *Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis* and *Enterococcus faecium* Causing Urinary Tract Infections in Kidney Transplant Recipients over 8 Years: Single-Center Study. *Antibiotics (Basel)* **9**(6):284. doi: 10.3390/antibiotics9060284. PMID: 32466463; PMCID: PMC7344424.

[3] **Mirzayev F, Viney K, Linh NN, Gonzalez-Angulo L, Gegia M,** et al. (2021) World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. *Eur Respir J* **57**(6):2003300. doi: 10.1183/13993003.03300-2020. PMID: 33243847; PMCID: PMC8176349.

[4] **Santos-Aberturas J, Vior NM.** (2022) Beyond Soil-Dwelling Actinobacteria: Fantastic Antibiotics and Where to Find Them. *Antibiotics (Basel)* **11**(2):195. doi: 10.3390/antibiotics11020195. PMID: 35203798; PMCID: PMC8868522.

[5] **Khudhair SA, Ismael HA, Rashak NA, Mallooki AM, Ghafil JA, Zgair AK.** (2023) Antimicrobial effect of soil microorganisms ' products against different clinical bacterial isolates. World J Exp Biosci **11**: 41-45.

[6] **Jakubiec-Krzesniak K, Rajnisz-Mateusiak A, Guspiel A, Ziemska J, Solecka J.** (2018) Secondary metabolites of actinomycetes and their antibacterial, antifungal and antiviral properties. *Polish J Microbiol* **67**(3): 259–272. https://doi.org/10.21307/pjm-2018-048.

[7] **Nikolouli K, Mossialos D.** (2012) Bioactive compounds synthesized by non-ribosomal peptide synthetases and type-I polyketide synthases discovered through genome-mining and metagenomics. *Biotechnol Lett* **34**(8):1393-403. doi: 10.1007/s10529-012-0919-2. Epub 2012 Apr 6. PMID: 22481301.

[8] **De Simeis D, Serra S.** (2021) *Actinomycetes*: A Never-Ending Source of Bioactive Compounds-An Overview on Antibiotics Production. *Antibiotics (Basel).* **10**(5):483. doi: 10.3390/antibiotics10050483. PMID: 33922100; PMCID: PMC8143475.

[9] **Bhatti AA, Haq S, Bhat RA.** (2017) Actinomycetes benefaction role in soil and plant health. *Microb Pathog* **111**:458-467. doi: 10.1016/j.micpath.2017.09.036. Epub 2017 Sep 18. PMID: 28923606.

[10] **Norouzi H, Danesh A, Mohseni M, Rabbani Khorasgani M.** (2018) Marine Actinomycetes with Probiotic Potential and Bioactivity against Multidrug-resistant Bacteria. *Int J Mol Cell Med* **7**(1):44-52. doi: 10.22088/IJMCM.BUMS.7.1.44. Epub 2018 Apr 3. PMID: 30234072; PMCID: PMC6134418.

[11] **Fatahi-Bafghi M, Rasouli-nasab M, Yasliani-Fard S, Habibnia S, Gharehbaghi F, et al.** (2019). Diversity and antimicrobial activity of actinomycetes isolated from Lut Desert: the extremely arid climatic zones of Iran. Iran. *Int J Pept Res Ther* **25**:1201–1207 (2019). https://doi.org/10.1007/s10989-018-9767-9.

[12] **Yao H, Liu J, Jiang X, Chen F, Lu X, Zhang J.** (2021) Analysis of the Clinical Effect of Combined Drug Susceptibility to Guide Medication for Carbapenem-Resistant *Klebsiella pneumoniae* Patients Based on the Kirby-Bauer Disk Diffusion Method. *Infect Drug Resist* **14**:79-87. doi: 10.2147/IDR.S282386. PMID: 33469322; PMCID: PMC7812027.

[13] **Gurung TD, Sherpa C, Agrawal VP, Lekhak B.** (2010). Isolation and Characterization of Antibacterial Actinomycetes from Soil Samples of Kalapatthar, Mount Everest Region. *Nepal J Sci Technol* ***10***:173–182. https://doi.org/10.3126/njst.v10i0.2957.

[14] **Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, Al-Ouqaili MTS, Chinedu Ikem J,** et al. (2022) Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *J Clin Lab Anal* **36**(9):e24655. doi: 10.1002/jcla.24655. Epub 2022 Aug 10. PMID: 35949048; PMCID: PMC9459344.

[15] **Liu S, Sun C, Ha Y, Ma M, Wang N, et al.** (2024). Novel antibacterial alkaloids from the Mariana Trench-derived actinomycete Streptomyces sp. SY2255. *Tetrahedron Letters* **137***:*154935. https://doi.org/10.1016/j.tetlet.2024.154935.

[16] **Kazmierczak KM, Bradford PA, Stone GG, de Jonge BLM, Sahm DF.** (2018) In Vitro Activity of Ceftazidime-Avibactam and Aztreonam-Avibactam against OXA-48-Carrying Enterobacteriaceae Isolated as Part

of the International Network for Optimal Resistance Monitoring (INFORM) Global Surveillance Program from 2012 to 2015. *Antimicrob Agents Chemother* **62**(12):e00592-18. doi: 10.1128/AAC.00592-18. PMID: 30249690; PMCID: PMC6256811.

[17] **Galani I, Karaiskos I, Giamarellou H.** (2021) Multidrug-resistant *Klebsiella pneumoniae*: mechanisms of resistance including updated data for novel β-lactam-β-lactamase inhibitor combinations. *Expert Rev Anti Infect Ther* **19**(11):1457-1468. doi: 10.1080/14787210.2021.1924674. Epub 2021 May 24. PMID: 33945387.

[18] **El Zowalaty ME, Lamichhane B, Falgenhauer L, Mowlaboccus S, Zishiri OT,** (2023) Antimicrobial resistance and whole genome sequencing of novel sequence types of *Enterococcus faecalis*, Enterococcus faecium, and Enterococcus durans isolated from livestock. *Sci Rep* **13**(1):18609. doi: 10.1038/s41598-023-42838-z. Erratum in: Sci Rep. 2023 Nov 14;13(1):19879. doi: 10.1038/s41598-023-46976-2. PMID: 37903806; PMCID: PMC10616195.

[19] **Nazari MT, Machado BS, Marchezi G, Crestani L, Ferrari V,** (2022) Use of soil actinomycetes for pharmaceutical, food, agricultural, and environmental purposes. *3 Biotech* **12**(9):232. doi: 10.1007/s13205-022-03307-y. Epub 2022 Aug 19. PMID: 35996673; PMCID: PMC9391553.

[20] **Cuervo L, Méndez C, Salas JA, Olano C, Malmierca MG.** (2022) Volatile Compounds in Actinomycete Communities: A New Tool for Biosynthetic Gene Cluster Activation, Cooperative Growth Promotion, and Drug Discovery. *Cells* **11**(21):3510. doi: 10.3390/cells11213510. PMID: 36359906; PMCID: PMC9655753.

[21] **Sedeek AM, Ismail MM, Elsayed TR, Ramadan MA.** (2022) Recent methods for discovering novel bioactive metabolites, specifically antimicrobial agents, from marine-associated micro-organisms. *Lett Appl Microbiol* **75**(3):511-525. doi: 10.1111/lam.13728. Epub 2022 May 9. PMID: 35485872.

Author affiliation

1. Department of Biology, College of Science, Baghdad, Iraq.

ORCID:
Ghafil JA: https://orcid.org/0000-0003-1461-302X
Zgair AK: https://orcid.org/0000-0002-2356-3338