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Antibacterial Activity of Secondary Metabolites Produced by Saline Soil

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Abstract: **Objective:** The current study aimed to detect fungal isolates isolated from saline soil to produce active compounds with antimicrobial effects. **Methods:** Four types of fungi isolated from saline soils were obtained from different locations. The effectiveness of these fungal was tested against two types of gram-positive bacteria (Staphylococcus aureus and Streptococcus agalicae) as well as against two types of gram negative bacteria (Enterobacter sp.. and Pseudomonas sp.) by the method of diffusion in wells. **Results:** The results of the current study showed that four fungal isolates were obtained: Aspergillus niger, Aspergillus flavus, Penicillium notatum, and Bacillomyces sp. Symbols were given to the supernatant of each fungal isolate, and these symbols are AA, AB, AC, and AD, respectively. The results showed that the fungus Penicillium notatum produced secondary metabolites that have the ability to inhibit bacteria, especially gram-negative bacteria, while the fungus Aspergillus flavus was distinguished by producing secondary metabolites that have a more inhibitory effect on gram-positive bacteria. **Conclusion:** It can be concluded from the current study that it is possible to isolate microbes from the soil that have the ability to produce secondary metabolic compounds that are effective against Gram-positive and Gram-negative pathogenic bacteria.

Key points: Salaine Soil, Aspergillus flavus, Penicillium notatum, Antibacterial activity.

Introduction

Infection has been the main cause of diseases throughout human history, especially bacterial infections of microorganisms that pose a significant threat to human health and cause other acute deadly diseases [1,2]. Every year, millions of people suffer from these microorganisms. Infectious diseases primarily affect the elderly and the impoverished, particularly in developing nations where their daily activities expose them to harmful environments [3,4]. The extended disease brought on by the rise of drug-resistant diseases and the expense of treatment have caused their suffering to increase many times. Due to the annual rise in drug-resistant pathogens, estimates show that over 70% of pathogenic bacteria are resistant to at least one antibiotic, making the development of novel and potent antimicrobial medications imperative [5,6].

A major component of ecosystems and one of the most significant tools in the field of biotechnology, fungi are regarded as one of the largest groups of living organisms found worldwide[7,8]. Applications for fungi are numerous and include industry, agriculture, medicine, and the environment. The most significant application of fungus is the synthesis of secondary metabolites, such as antibacterial compounds, which can aid in medical therapy [9,10].

Antibiotics are, of course, antimicrobial compounds that are created by the secondary metabolic processes of some microorganisms, including bacteria, fungi, and actinomycetes. These agents are toxic to other microorganisms and have the ability to suppress or even eradicate them [11]. Through various methods of action, secondary metabolites are utilized to treat infections brought on by bacteria and other organisms that can infect humans and animals and cause sickness [12]. Due to

their structural nature and the degree of their binding to specific target sites within bacteria, they have been classified as follows: cell wall synthesis inhibitors (penicillin, bacitracin, and vancomycin), cell membrane function inhibitors (polymyxin B and colistin), protein synthesis inhibitors (aminoglycosides, chloramphenicol, and tetracycline), and nucleic acid synthesis inhibitors (quinolones, metronidazole, and rifampicin)[13,14]. Antibiotics can be found from a wide range of possible sources, including soil and medicinal herbs. However, the majority of researchers continue to focus their efforts on soil, a naturally occurring mixture of organic and mineral particles, in an attempt to find novel antibiotics with medicinal potential [15,16,17].

Small organic compounds known as secondary metabolites are created by living things and are not necessary for their growth, development, or reproduction. In order to occupy the proper niche and acquire access to food, they also contribute to antagonism, competition, and defense mechanisms against other organisms. Based on their chemical makeup, these substances are divided into four major groups: terpenoids, alkaloids, glycosides, and phenols[18]. Secondary metabolites from microorganisms have shown to be a valuable resource for medication development. Strong antibiotics that were separated from microbial nutrition broth were later discovered as a result of the discovery of penicillin. Additionally, the necessity to find novel pharmaceutical chemicals is important because the number of drug-resistant microbes is continuously rising [19,20].

Materials and Methods

Collecting samples

A spoon was used to collect five soil samples from various sites within the drainage ditches of Karbala, Hindia, at a depth of 10 cm. The samples were then transferred into sterile 200 ml glass containers. The microbiology research laboratory of the Department of Life Sciences, College of Pure Education Sciences, University of Karbala, Iraq, received the classified soil sample.

Isolation and identification of fungal isolates:

Following a series of tenfold dilutions, fungal isolation was carried out on agar plates using the pour plate technique. Nine milliliters of sterile distilled water were added to a 1-gram sample of dirt, and the mixture was thoroughly stirred.

Using potato dextrose agar supplemented with 50 micrograms/ml of streptomycin to inhibit bacterial growth, 0.1 ml of the third and fourth dilutions were inoculated in duplicate onto PDA media after a series of tenfold dilutions. To promote fungal development, the medium's pH was also brought down to 5.8. After 96 hours of incubation at room temperature (28 ºC), the individual fungal colonies were taken out of the plates and repeatedly sub cultured until pure cultures were achieved [21].

Using suitable media, cultures, slides, and the most recent classification keys from pertinent sources, the isolated fungi were identified at the genus and species levels based on morphological characteristics (as the colonies were examined for slow or fast growth, colony surface (flat, piled, regularly or irregularly folded), texture (yeast-like, powdery, granular, velvety, or cottony), pigmentation (whether superficial or reverse), and fine morphological characteristics (hyphae, large conidia, small conidia, chlamydospores, and other specific fungal structures) [22,23].

Inoculation of the medium with the fungal isolate:

The Heart Brain Infusion Agar medium was sterilized in the autoclave at 121 ºC for 60 minutes. Each test tube containing 10 ml of sterile medium was inoculated under sterile conditions with the pure fungal colony in PDA medium. The inoculated media were kept at room temperature (27-28 °C) with a pH of 5.8 for 7 days in incubation. After incubation, the culture media were centrifuged using a centrifuge at 4000 RPM for 30 minutes. Following that, supernatant was evaluate using the agar well diffusion method [24].

Bacterial activation

Four types of bacteria were obtained from the main laboratory at Al-Husseini Hospital in Karbala Governorate, two types of Gram-positive bacteria: *Staphylococcus aureus* and *Streptococcus agalactiae*. In addition to two types of Gram-negative bacteria: *Pseudomonas* sp., *Enterobacter* sp. The test bacteria are activated in Mueller-Hinton broth half an hour before being cultured.

Antimicrobial efficacy test

After activating the bacteria in the liquid Mueller-Hinton medium, filter paper discs (6 mm diameter) are sterilized using an autoclave and immersed in each solution of the crude fungal extract for 5 minutes. After spreading using a bacterial spreader, the filter paper discs containing the extracts are put on top of the Mueller-Hinton agar medium in Petri dishes, to which 0.2 ml of the pathogenic bacteria's bacterial suspension is added. Inhibition zones surrounding the filter paper disc show the biological activity of the crude secondary metabolites generated by the tested fungal isolates after the plates were incubated for 24 hours at 37 ºC [24,25].

Statistical analysis

A 5×2 factorial experiment analysis with 6 replications was part of the statistical analysis. The bacterial species and fungal supernatant were the factors that were examined. At a probability level of 0.05, the Least Significant Difference (LSD) test was also used to determine whether the mean differences were significant [26].

Results and Discussion

Four types of fungi were identified from all soil samples, and these types are *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus niger*, and *Bacillomyces* sp. A symbol was assigned to each supernatant for all fungal isolates as shown in the table.

This study focused on evaluating the antibacterial efficacy against pathogenic strains of This study focused on evaluating the antibacterial effectiveness against pathogenic strains of Gram-positive bacteria for *Streptococcus agalactiae* and *Staphylococcus aureus* and Gram-negative bacteria for *Streptococcus* sp. and *Enterobacter* sp attempt obtaining a therapeutic antibiotic from extracts of salt marsh fungi.

Table (1): Fungal strain codes for fungi isolated from saline soil.

Innate isolation	The candidate's code
Aspergillus niger	AA
Aspergillus flavus	AB
Penicillinum notatum	AC
Bacillomyces sp.	AD

The results of Table (2) indicate that the best fungal extracts in their antibacterial activity against Gram-negative bacteria is the AC extract of the fungus *Penicillium notatum*, with inhibition diameters of 4.1 and 3.2 mm for *Enterobacter* sp. and *Pseudomonas* sp. respectively. and the bacteria *Pseudomonas* sp. Respectively as the efficacy of this extract was comparable to that of the standard antibiotic used here in this experiment. It is worth noting that the bacteria *Enterobacter* sp.

They are more sensitive to fungal extracts.

She is more sensitive to fungal pathogens. *Pseudomonas* sp is a type of bacteria.

Table 2: Effectiveness of fungal filtrates against Gram-negative bacteria measured by inhibition diameters in mm.

Morale level P value	Gram-negative pathogenic bacteria		Fungal exudate
	Pseudomonas sp.	*Enterobacter* sp.	
0.820	0.8 ± 4.6	0.7 ± 4.2	Antibiotic

0.394	0.6 ± 2.2	0.4 ± 1.7	(*Aspergillus niger*) AA
* 0.048	0.7 ± 1.9	0.8 ± 2.3	(*Aspergillus flavus)* AB
* 0.039	0.5 ± 3.2	1.1 ± 4.1	(*Penicillinum notatum)* AC
* 0.044	0.3 ± 1.2	0.4 ± 1.9	*(Bacillomyces* sp.) AD
	*** 0.007**	*** 0.032**	Morale level **(P value)**
	1.091	**1.723**	Less of a moral difference **(LSD)**

The numbers indicate the mean ± standard deviation.

* Indicates the presence of a significant difference

The results of the statistical analysis in Table 3 indicate that there are significant differences (P<0.05) in the inhibition zone diameter of fungal supernatant against both types of Gram-positive bacteria, with the *P value* for *S. aureus* being 0.0391 and for *Streptococcus agalactiae* being 0.0407. It can also be noted that S. aureus bacteria were more sensitive than *Streptococcus agalactiae* bacteria to the AB supernatant, with inhibition diameters of 4.4 mm and 3.9 mm, respectively.

Table 3: Effectiveness of fungal filtrates against Gram-positive bacteria measured by inhibition diameters in mm.

Morale level P value	Pathogenic Gram-positive bacteria		Fungal exudate
	*Streptococcus agalica*e	*Staphylococcus aureus*	
0.179	1.1 ± 4.1	0.8 ± 4.5	Antibiotic
0.207	0.4 ± 3.2	0.6 ± 3.7	(*Aspergillus niger*) AA
* 0.042	0.8 ± 3.9	1.2 ± 4.4	(*Aspergillus flavus)* AB
0.073	0.4 ± 2.2	0.3 ± 2.6	(*Penicillinum notatum)* AC
0.081	0.5 ± 1.9	0.2 ± 1.7	(*Bacillomyces* sp.) AD
	*** 0.0407**	*** 0.0391**	Morale level **(P value)**
	2.410	**1.108**	Less of a moral difference **(LSD)**

The numbers indicate the mean ± standard deviation.

* Indicates the presence of a significant difference

The results of this study revealed the presence of fungi in saline soil capable of producing secondary metabolites with antibacterial properties. The four fungal isolates from the saline soil showed an inhibitory effect against both Gram-positive and Gram-negative bacteria. The high rate of antibiotic production may be related to environmental factors and conditions (salinity, pH, temperature), where these secondary metabolites act as a defensive mechanism for the fungi and microbes that produce them to maintain their presence in this environment or enable them to invade another environment.

While some studies' findings showed an inhibitory role comparable to the one found here, other studies' findings showed values that were either higher or lower. According to a study by the Adelaide group (2011), 23% of the 119 fungal isolates that were taken from soil sources were efficient against harmful bacteria [27].

In 1998, Ivanova and his team found that 26% of the 491 bacteria they obtained from different marine sources had action against the test bacterium [28].

According to Ivanova and his team (1998), out of 491 bacteria isolated from different marine sources, 26% of the isolates exhibited action against the test microorganisms [29].

In addition to the agreement between the results of our current study and those reached by Al-Daamy and his group (2012) in their study, which The inhibitory activity of the isolated fungal strains against three Gram-positive bacterial species and three Gram-negative bacterial species [30].

Conclusions

Through the results of the current study, the following conclusions can be reached:

1. Some types of salt-tolerant soil fungi can be isolated that have the ability to produce compounds with antibacterial activity against pathogenic bacteria.
2. Gram-positive bacteria were more sensitive to the secondary metabolites produced by Penicillium notatum fungus.
3. Gram-negative bacteria are the most sensitive to the secondary metabolites produced by the fungus *Aspergillus flavus*.

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