

Comparison of the Bioeffect of Different Locally Isolated Antifungal Bacteria

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الخلاصة

جمعت ثلاثة عشر عينة تربةٍ مختلفةٍ من المنطقة القريبة لمنطقة rhizosphere من أماكنٍ مختلفةٍ في مدينة الموصل/ العراق. عزل من هذه العينات ٦٦ عزلة بكتيرية مختلفة وبعد اختبار قابلية كل عزلة في تثبيط نمو عدد من الفطريات تم الحصول على ٧ عزلات بكتيرية مختلفة لها القدرة على تضاد الفطريات رموزها AF1 - AF7. تم تشخيص هذه العزلات، وتبين أنها عائدة لعزلات بكتيرية مختلفة موجبة وسالبة لصبغة كرام مثل *Pseudomonas*، *Bacillus* و *Paenibacillus*. كذلك تم مقارنة الفعاليات ضد الفطرية لهذه العزلات ضد الفطريات *Aspergillus*، *Alternaria*، *Penicillium*، *Fusarium*، وأظهرت النتائج بان هناك احتمالية كبيرة لعزل بكتريا مضادة للفطريات من المنطقة البعيدة عن الرايزوسفير (البعيدة عن جذور النباتات) non-rhizosphere soils (ومن الممكن استخدام هذه العزلات للمقاومة الحيوية بدلا من الكيماويات الضارة) وان العزلات AF3 و AF7 (والتي هي من نوع Gram positive) كانتا أكثر نشاطا في تضادها للفطريات بسبب النسب العالية لقطرها التثبيطي inhibition zone ضد الفطريات وهذه العزلات عائدة لجنسي *Paenibacillus* و *Bacillus* على التوالي وان سبب التثبيط هو إنتاج مواد مثبطة للفطريات أو مضادات حيوية antibiotics.

ABSTRACT

Thirteen different soil samples near the rhizosphere were collected from different places in Mosul city/ Iraq. Sixty six different bacterial isolates were isolated from these samples. After testing the capability of each one of these isolates in antagonizing some pathogenic fungi we had 7 different bacterial isolates with an antifungal activities. They were designated as AF1-AF7. An identification of these bacteria was done, and

they appeared to belong to different Gram positive and Gram negative bacteria such as *Bacillus*, *Pseudomonas*, and *Paenibacillus*. The antifungal activities of these bacteria were compared between each other on the pathogenic fungi *Fusarium*, *Penicillium*, *Alternaria*, and *Aspergillus*. The results showed that there is a high probability of isolating different potent antifungal bacteria from the non root-associated soils (and they could be used as potent biological control agent instead of harmful chemicals). The isolates AF3 and AF7 were the most active isolates against the test fungi because of their high inhibition zones and they were belonged to Gram Positive bacteria *Paenibacillus* and *Bacillus* respectively, and the inhibition was due to the production of antifungal metabolites or antibiotics.

Introduction

Most of the important cereal crops in the world are prone to many soil-borne plant pathogens that reduce the quality and quantity of grain production (1). Fungi are the primary causes of grain loss, and some of them produce compounds that are toxic when consumed (2). Fungal diseases of plants are usually controlled by some combination of cultural practices, including the use of fungicides, and increasing the plant host resistance. Fungicides are the primary means of fungal disease control, but their use is currently controversial because investigation have indicated potentially undesirable environmental side effects (3).

Due to environmental concerns, there is a considerable interest in finding alternatives to chemical pesticides for suppression of soil borne plant pathogens. Biological control represents an attractive alternative for the future because of the many concerns about the pesticides use. Ideally, an agent of biological control of fungal root pathogens should exert a sufficient amount of antagonistic activity in the rhizosphere to significantly reduction in root disease symptoms (4).It was mentioned by many researches that the soil, especially the rhizosphre, contains different kinds of bacteria (Gram positive and Gram negative) that positively affect the plant, especially by antagonizing the soil borne plant pathogenic fungi (5,6,7,and 8). According to this we aimed in this study to isolate (and to know) different types of bacteria from the non-rhizosphere soils that have antifungal activities toward different plant and human pathogenic fungi (they might be used as good biological control agent) and to compare the antifungal activity between each one of these bacteria.

Materials and Methods

Test fungi:

The Plant pathogens *Penicillium nalgiovense*, *Fusarium graminearum*, *Alternaria alternata*, and one human fungal pathogen *Aspergillus niger* were obtained from Biology Department/ College of Science/University of Mosul/Iraq.

Collection of samples:

Thirteen different soil samples (garden soils) were collected from different places in Mosul city, garden soil samples of Al-Faesalia region (3 samples), Al-Zuhoor region (3 samples), Al-Muthana region (4 samples), and garden soils of Mosul University (3 samples). The soil samples were taken from places that are near the rhizosphere of garden plants (the non root-associated soil samples).

Isolation of bacteria:

One gram of each soil sample was putted in 9ml of sterilized distilled water then serial dilutions were done for each soil sample, and 0.1 ml of the dilutions 10^{-4} and 10^{-5} of each sample was cultured on 9cm Petri dish containing nutrient agar and incubated at 30 °C for 24 hour. The effect of the growing colonies were tested against test fungi separately. The antifungal bacterial isolates were subcultured on nutrient agar slants for identification in which several morphological, physiological, and biochemical tests were done to identify these bacteria and their effects were tested against different pathogenic fungi.

Antifungal bioassay test :

In order to examine the antagonistic properties of bacterial isolates against phytopathogenic fungi, and according to Kobayashi *et al.*,2000 and Gupta *et al.*,2001 (9 and 10), an agar block (5 mm diameter) of 5-day-old culture of fungal pathogen was placed in the centre of plate containing potato dextrose agar (PDA) (or Sabouraud dextrose agar in the case of human pathogenic fungi) (5). A loopful of 24-h-old culture of the each of the isolated bacterium was inoculated at 2 cm juxtaposed to the pathogen. Some plates were left without inoculation of the bacteria to serve as a control. The plates were incubated at $28 \pm 1^{\circ}\text{C}$ for 5 days and inhibition of fungal growth was measured.

Results and Discussion

Isolation of antifungal bacteria:

From thirteen different soil samples that were taken from different places in Mosul city, sixty six different bacterial isolates were obtained. Each one of these bacterial isolates was preliminary tested against the fungus *Alternaria alternata* .Seven different bacterial isolates showed an antifungal activities at first toward *Alternaria alternata* and later when tested against other fungi, and they were designated as AF1, AF2, AF3, AF4, AF5, AF6, and AF7.

Identification of bacterial isolates:

The antifungal bacterial isolates were subjected to several morphological, physiological, and biochemical tests to identify them (11 and 12), and the results were as follow:

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AF1, AF2, AF4, AF5, and AF6 isolates were identified as *Pseudomonas* spp.

AF3 and AF7 were identified as *Paenibacillus* spp. and *Bacillus* spp. respectively.

Antifungal bioassay test:

The seven bacterial isolates that preliminary showed antagonistic or antifungal activities were tested against different phytopathogenic fungi and against the human fungal pathogene *Aspergillus niger*. The isolates were ranked depending on the diameter of inhibition zone for fungi that caused by each isolate. The results are illustrated in (table 1) (see also figure 1 and figure 2):

Table (1): The bioassay test of the antifungal isolates

Bacterial Isolates	Inhibition Zone (mm) on:			
	<i>Fusarium-graminearum</i>	<i>Penicillium-nalgiovense</i>	<i>Alternaria-alternata</i>	<i>Aspergillus-niger</i>
AF 1	2	0	5	0
AF 2	2	0	4	0
AF 3	5	5	7	4
AF 4	0	0	6	0
AF 5	2	0	6	0
AF 6	3	0	6	0
AF 7	6	4	7	3

Form the above results, it is obvious that the antifungal bacterial isolates showed different antagonistic activities toward the selected pathogenic fungi ,and this might be due to the different capabilities of each isolates to antagonize the different fungi, and also this might be due to the different antifungal metabolites that were produced and secreted by the different bacterial isolates (13). Many researches mentioned that the majority of existing biocontrol agents for management of soil-borne diseases were isolated from the rhizosphere(7). Also there is a possibility to explore antagonists from other habitats (such as non-rhizosphere soils, internal plant habitat, and seed surfaces) as potent biocotrol agents. This reflects our results in finding different antifungal bacterial species in the non-rhizosphere soil samples (14). *Pseudomonas* spp. are effective root colonizer and biocotrol agent by its production of antibiotics and other antifungal metabolites including hydrogen cyanide and siderophores (15, 16). Several *Bacillus* strains (such as *B. cereus* and *B. subtilis*) can protect plants from pathogens, and it is known that they produce several antibiotics and they are often found in soil and are associated with plants (17 and 18). Also it is well known, especially in recent years, that many species of the Gram positive *Paenibacillus* bacteria showed in vitro

antagonistic activities against several fungal phytopathogens belonging to the ascomycetes, basidiomycetes, and oomycetes (19). From all above, it seems that our results were in agreement with many researchs in that different kinds of bacteria that are naturally inhabitant in the non-rhizosphere soils (such as *Pseudomonas*, *Bacillus*, and *Paenibacillus*) have an antagonistic activities against various phytopathogenic fungi and human pathogenic fungi and can be used as biocontrol agents. This activity may differ from one species to another depending on the nature of the antifungal organism(or the nature of the antifungal compound) or the circumstances and conditions that surround that organism. The results showed that Gram positive *Paenibacillus* and *Bacillus* spp. are more effective in antagonizing pathogenic fungi than Gram negative *Pseudomonas* spp. because they showed the higher inhibition zones toward the fungi, and that is in agreement with Danielsson *et al.*,2007 (20). Also in all above cases, the results showed that the best conditions for the production of the antifungal compound(s) from the isolated *Pseudomonas*, *Paenibacillus*, and *Bacillus* spp. was incubation at 28⁰C for 5 days and these are in agreement with Gupta *et al.*, (2001) and Lee *et al.*, (2008) (10 and 19).

Thus it was concluded that the non-rhizosphere soils may contain different potent antifungal bacterial growth. The Gram positive bacteria might be the most effective biocontrol agent, and their antagonistic activity was due to the production of antifungal metabolites.

Also it is recommended to make another study to compare the bioeffect of the different isolated antifungal bacteria from the non-rhizosphere, rhizosphere, rhizoplane and other different soil samples.



Figure(1): The bioeffect of the isolate AF3 on *Alternaria alternata*.



Figure(2): The bioeffect of the isolate AF7 (left) and of AF6 (right) on *Fusarium graminearum*.

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