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Isolation four species of *Bacillus* and tested activity against some filamentous fungi

Mohammed Abaas Mahdi

Basrah University, College of Science, Biology Department

ABSTRACT

During study isolated 4 species of *Bacillus* from 10 soil samples Al-Hartha soil by using dilution method. Tested inhibition ability these species on growth five filamentous fungi. The species *B. stearothermophilus* appeared inhibition effect on the all tested species, while the *B. subtilis*, *B. polymyxa* showed inhibition effect on the all species tested excepted *Gymnoascella dankalensis* while the *B. licheniformis* appeared effect just on the *G. dankalensis*.

Key words: Antifungal, Zone diameters in millimeters. Gram positive Rod shaped, *Bacillus sp*, preliminary screening.

INTRODUCTION

Microorganisms produces many antibiotics but few from it safety for medical using backed to product this antibiotics for many limited genera examples: *Streptomyces*, *Penicillim*, *Micromonaspara*, *Bacillus* [1]

Genus *Bacillus* back to the family *Bacillaceae*, which graded below 37 genera which forming Endospore [2].

Many types of genus *Bacillus* Produce many important compounds such as enzymes Protease [3] and antibiotics example *Bacillus pumilus* and *Bacillus subtilis* have the ability to produce many antibiotics peptide such as : Tyrocidin, gramicidin, polymyxinbacitracin ciltutin[4,5]

Most antibiotics which product from genus *Bacillus* appeared inhibited activity against gram positive and negative While Polymyxin, cirulin, chaetomacin, colistin appear antifungal activity against yeasts and filamentous fungi such as *Aspergillus*, *Rhizopus*, *Gymnoascella*. [6]

Therefore, the current study aimed to isolate some species of the genus *Bacillus* and perform a preliminary survey to determine the inhibitory capacity of fungi

MATERIALS AND METHODS

Bacterial isolates

10 soil samples taken from the area of Hartha and weight of each and every one of them (1 g) and he underwent a series perform Serial dilution start (10^{-1}) to (10^{-10}), planted all dilutions on the Petri dishes contained nutrient agar then incubated at 37 C for 24 hours.

Diagnosis bacterial isolates depended on [7]

Fungal isolates used

Was obtained fungal isolates from fungi Research Laboratory in the Department of Biology / College of Science / University of Basra have been diagnosed by an assistant professor Najwa Mohammed Jamil Ali Abu-Mejdad

Preliminary survey of the effect of some bacterial isolates against some filamentous fungi in vitro and in vitro

Used Agar well diffusion method by work wells on the solid culture medium Sabourauds Dextrose Agar (SDA) depended on methods [8] as the follow :-

1-prepared sabourauds dextrose agar and active fungal isolates used this medium at a temperature of 27°C for a week.

2-Prepared fungal suspensions from five fungal isolates and a concentration of 2 of Standard McFarland scale has been spreader on the SDA by use of sterile L-shap.

3-Standard McFarland scale [9] Consists of a mix of different sizes:

Table (1): a mix of different sizes of sulfuric acid and Barium chloride

Tubes number	BaCl ₂ (ml)	H ₂ SO ₄ (ml)	10× ⁶ /ml
1	0.1	9.9	3
2	0.2	9.8	6
3	0.3	9.7	9

4-work wells a diameter of 6 mm and for each culture media using sterile cork borer add 100 Microliter of bacterial isolates stimulant on medium nutrient agar concentration was prepared first scale McFarland and placed in the wells and using Micropipette with sterile lids and with extreme caution to avoid scattering of bacterial isolates suspension on the culture medium.

5-fungi incubated at 27°C for a week and then measured inhibition zone by mm

RESULTS AND DISCUSSION

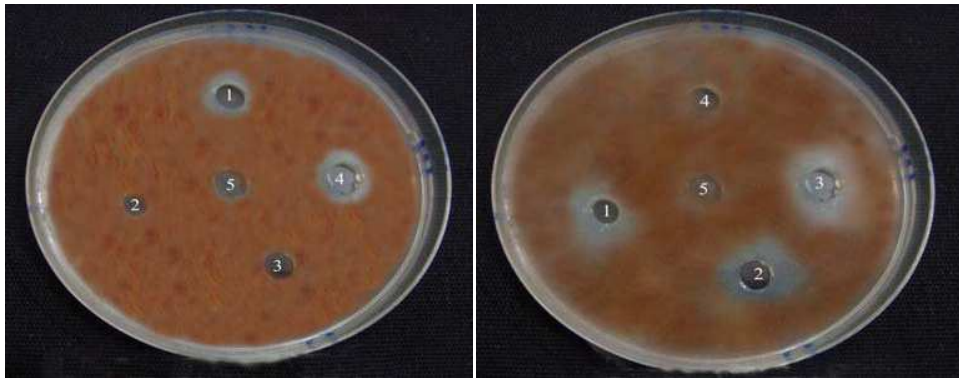
The results showed isolates belonging to the genus *Bacillus* and the test results were as shown in the table below:

Table (2): shows the results of initial tests on the diagnosis species

Isolates number	Tests	amylase	VP	citrate	Growth At 55 C	Gram staining	oxidase	catalase	Spore formation	Motility
<i>Bacillus stearothermophilus</i>		+	-	-	-	+	+	+	+	+
<i>B.polymyxa</i>		+	+	-	-	+	+	+	+	+
<i>B.subtilis</i>		+	+	+	-	+	+	+	+	+
<i>B.licheniformis</i>		+	+	+	+	+	+	+	+	+

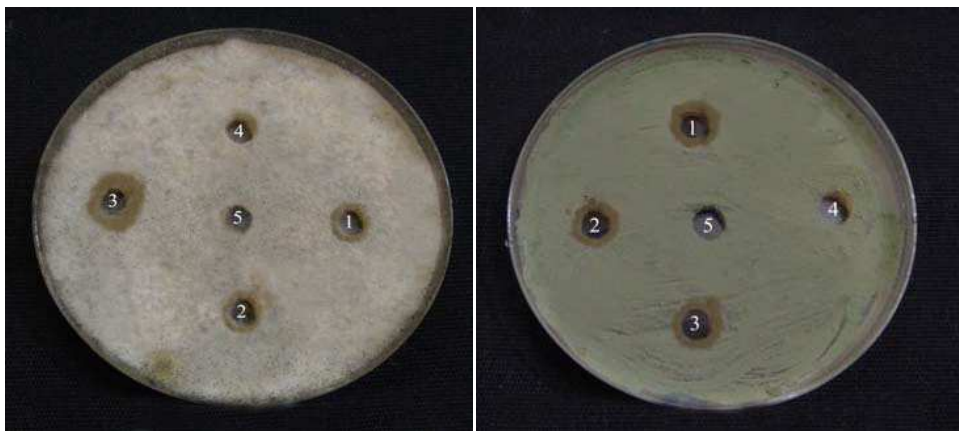
Table(3): Inhibition zone rates scaled by mm for antifungal activity to four bacterial isolates backed to *Bacillus sp*

Fungal isolates	Inhibition zone rates scaled by mm				
	<i>Bacillus stearothermophilus</i>	<i>B.polymyxa</i>	<i>B.subtilis</i>	<i>B.licheniformis</i>	control
<i>Aspergillus niger</i>	8	10	10	0	0
<i>A.flavus</i>	10	8	10	0	0
<i>A.nidulans</i>	8	10	10	0	0
<i>Rhizopus stolonifera</i>	10	8	15	0	0
<i>Gymnoascella dankalensis</i>	10	0	0	8	0



Aspergillus flavus

Aspergillus niger



Gymnoascus dankalensis

Aspergillus nidulans



Rhizopus stolonifera

Plate (1): diameters rates of inhibition measured by (mm) of the antifungal activity four bacterial isolates belonging to the genus *Bacillus* sp

1=*Bacillus stearothermophilus* 2= *B. polymyxa*, 3= *B. subtilis*, 4= *B. licheniformis*

Appeared isolation ratio in the current study insulation 100 % 4 types out of 10 isolates showed the antifungal activity used in the test and this percentage rate convergence that have emerged in the study[6] which recorded a 9 types of insulation producer out of 12.

Effectively isolates showed a clear filamentous fungi and this is consistent with what mentioned [10] that most of the antibiotics produced by *Bacillus* work against fungi which suggested the peptide antibiotics product by genus *Bacillus* interact with plasma membrane and cell walls[3] for example bacitracin prevent cell wall formation of fungi[11,12]

While the results show a table (2) plate (1) the effectiveness of genera *Bacillus* towards five species of filamentous fungi that these compounds have effective anti-fungal, and characterized by its ability to unite with protein cell and deposition Climate of nature and acts as a solvent good material fatty acids, it analyzes membranes of living cells and consequently graduated components intracellular exit out and the fungal cell die . [13,14]

CONCLUSION

The suspension of bacterial isolate *Bacillus subtilis* were revealed that optimum antifungal activity against *Gymnoascus dankalensis* The mean zone of antifungal activity for this isolate 15 mm for *Bacillus subtilis* and the other species also appeared antifungal activity but less in mean zone there for therefore this study possible to be encouraging to treat fungal that cause different infections Tutorials as it marks the beginning of extraction and purification of antibiotics produced by a species listed

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