Antimicrobial Activities of Certain Bacteria Isolated from Egyptian Soil Against Pathogenic Fungi


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Abstract: One hundred bacterial isolates were isolated from six different soil samples collected from Egypt. Twenty isolates could antagonize the selected human and plant pathogenic fungi with varying degrees. Out of them, only three were found to have remarkable inhibitory effect on all the tested pathogenic fungi. These bacterial isolates were identified as Tsukamurella inchonensis, Corynebacterium nitrilophilus and Cellulosimicrobium cellulos. The factors affecting the production of antifungal compounds reveal that glucose and potassium nitrate were the best carbon and nitrogen sources respectively. In addition, temperature (37°C), pH 7.0 and shaking conditions increase the production of antifungal compounds.

Key words: Antimicrobial activities, bacteria, pathogenic fungi, soil

INTRODUCTION

The antibiotics produced by bacteria have been gaining importance by many investigators. Bacillus species producing antibiotics have been used as biocontrol agents against pathogenic fungi[19,24]. Magnusson et al.[16] mentioned that more than 1200 isolates of lactic acid bacteria isolated from different environments were screened for antifungal activity, from which approximately 10% represent inhibitory activity and 4% showed strong activity against Aspergillus fumigatus. Biosynthesis of antibiotics is often regulated by carbon and nitrogen sources. El-Banna[19] found that antimicrobial substances produced by bacterial species were greatly influenced by variation of carbon sources. Galactose and glucose strongly enhanced the antimicrobial activity of Corynebacterium kutscheri and C. xerosis respectively, while ribose and lactose repressed their activity.

Several abiotic factors such as oxygen and temperature have been identified to influence antibiotic production from bacteria[20]. Jesper and Johan[12] studied the production of antifungal compounds from lactobacillus corynformis and mentioned that maximum activity was observed at pH values between 3.0 and 4.5, but it decreased rapidly when pH was adjusted to a level between 4.5 and 6.0 and was lost at higher pH values. Patelvo et al.[19] emphasized that the production of antifungal compounds from Bacillus licheniformis increased 30 fold when shake at 150 compared with 100 rpm.

This study is an attempt to investigate the antimicrobial activity of certain soil bacterial isolates recovered from Egyptian soil against some pathogenic fungi. Moreover, identification of the most active isolates was done.

MATERIALS AND METHODS

Soil Samples and Isolation of Bacterial Isolates: Six different soil samples were collected from three localities at Sharkiya and Qalubiya Governorates, Egypt for bacterial isolation according to the method of Crawford et al.[11].

Screening the Bacterial Isolates for Their Antagonistic Activities Against the Tested Fungi: The bacterial isolates were examined for their ability to produce antifungal compounds against human pathogenic fungi which isolated from patients and identified at Central Laboratory, Ain Shams University Hospital, Egypt (Aspergillus flavus, Aspergillus terreus and Aspergillus niger) Plant pathogenic fungi were isolated from infected plants and identified at Microbiology Department, Faculty of Science, Ain Shams University, Egypt (Fusarium oxysporium, Penicillium digitatum and Alternaria solani). The determination of the inhibitory effect of the bacterial isolates on tested pathogenic fungi was carried out according to agar-well diffusion method[11].
Identification of Bacterial Isolates: The active bacterial isolates were identified according to Bergey's manual of systematic bacteriology in Krieg and Holt[15] and Sneath et al.[23]. Biolog metabolic fingerprint was used to confirm the identification of the most active bacterial species.

Factors Affecting the Production of Antifungal Compounds: Carbon Sources: Glucose of modified fish meal extract broth[12] was substituted in equimolar by five different carbon sources, namely fructose, sucrose, lactose, starch and glycerol in order to obtained the most suitable carbon source for antifungal compounds production.

Nitrogen Sources to Find out the Best Nitrogen Source: for antifungal compound production, five different nitrogenous compounds in equimolar substituted potassium nitrate of modified fish meal extract broth such as ammonium sulphate, peptone, arginine, glutamic acid and were used.

Table 1: Antagonistic activity of bacterial isolates against human-and plant-pathogenic fungi

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<tr>
<th>Bacterial isolates</th>
<th>Human-pathogens</th>
<th>Plant-pathogens</th>
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<td>A. flavus</td>
<td>A. terreus</td>
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<td>5-9</td>
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Temperature: The effect of temperature was tested by using different incubation temperature (i.e., 25, 30, 37, 45 and 50 °C).

pH Value: The culture media were prepared with different range of pH values from 5 to 9 which adjusted by 0.1 N of NaOH and HCl.

Aeration: The medium was incubated at static and shaking conditions in order to find its effect on antifungal compounds production.

RESULTS AND DISCUSSION

Results: Isolation and Screening the Bacterial Isolates for Antagonistic Activities Against the Tested Fungi: One hundred bacterial isolates were obtained from soils collected from the three localities. Only 20 isolates showed antagonistic activities of variable degrees against all or some of the six tested human-and plant-pathogenic fungi as shown in Table (1). According to
Table 1: Continued

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Fig. 1a, b: a: Effect of different carbon sources on the antagonistic activity of Tsukamurella inchonensis against human-pathogenic fungi.

b: Effect of different carbon sources on the antagonistic activity of Tsukamurella inchonensis against plant-pathogenic fungi

Fig. 2a, b: a: Effect of different carbon sources on the antagonistic activity of Corynebacterium nitroliophilus against human-pathogenic fungi.

b: Effect of different carbon sources on the antagonistic activity of Corynebacterium nitroliophilus against plant-pathogenic fungi

Fig. 3a, b: a: Effect of different carbon sources on the antagonistic activity of Cellulosimicrobium cellulosans against human-pathogenic fungi.

b: Effect of different carbon sources on the antagonistic activity of Cellulosimicrobium cellulosans against plant-pathogenic fungi
Fig. 4a, b: 

(a) Effect of different nitrogen sources on the antagonistic activity of Tsukamurella inchonensis against human-pathogenic fungi

(b) Effect of different nitrogen sources on the antagonistic activity of Tsukamurella inchonensis against plant-pathogenic fungi

Fig. 5a: Effect of different nitrogen sources on the antagonistic activity of Corynebacterium nitriphilus against human-pathogenic fungi

Fig. 5b: Effect of different nitrogen sources on the antagonistic activity of Corynebacterium nitriphilus against plant-pathogenic fungi
Fig. 6a, b: a: Effect of different nitrogen sources on the antagonistic activity of Cellulosimicrobium cellulans against human-pathogenic fungi  
b: Effect of different nitrogen sources on the antagonistic activity of Cellulosimicrobium cellulans against plant-pathogenic fungi

Fig. 7a, b: a: Effect of different temperature degrees on antagonistic activity of Tsukamurella inchoensis against human-pathogenic fungi  
b: Effect of different temperature degrees antagonistic activity of Tsukamurella inchoensis against plant-pathogenic fungi

Fig. 8a, b: a: Effect of different temperature degrees on antagonistic activity of Corynebacterium nitrophiilus against human-pathogenic fungi  
b: Effect of different temperature degrees on antagonistic activity of Corynebacterium nitrophiilus against plant-pathogenic fungi
Fig. 9a, b: a: Effect of different temperature degrees on antagonistic activity of Cellulosimicrobium cellulans against human-pathogenic fungi
b: Effect of different temperature degrees on antagonistic activity of Cellulosimicrobium cellulans against plant-pathogenic fungi

Fig. 10a, b: a: Effect of different pH-values on the antagonistic activity of Tsukamurella inchoensis against human-pathogenic fungi
b: Effect of different pH-values on the antagonistic activity of Tsukamurella inchoensis against plant-pathogenic fungi

Fig. 11a, b: a: Effect of different pH-values on the antagonistic activity of Corynebacterium nitrilophilus against human-pathogenic fungi
b: Effect of different pH-values on the antagonistic activity of Corynebacterium nitrilophilus against plant-pathogenic fungi
their antagonistic activities on the six tested fungi, the isolates were grouped into four categories. The first category included eight isolates capable of antagonizing all the tested fungi. The second included seven isolates antagonize five of the tested fungi, the third comprises of three isolates that showed antagonistic effect on four of the pathogenic fungi, while the fourth category included two isolates capable of antagonizing three of the six tested fungi. The three isolates that showed most remarkable antagonistic activities against all the tested pathogenic fungi (No. 2-6, 3-20 and 2-15) were selected for further studies.

**Identification of Bacterial Isolates:** According to Bergey's manual of systematic bacteriology, seventeen of antagonistic bacterial isolates were identified to the genus *Bacillus*. The three strongest antagonistic bacterial showed different genera were further identified to the species level using Biolog metabolic fingerprint. They identified as *Tsukamurella inchonensis* (No. 2-6), *Corynebacterium nitrophiilus*, (No. 2-15), and *Cellulosimicrobium cellulosans* No. 3-20).

**Factors Affecting the Production of Antifungal Compounds:** Media without carbon source showed minimal antifungal compounds production for the three selected isolates (Figs. a & b in 1, 2 and 3). Moreover, glucose was the best carbon source followed by fructose, glycerol, sucrose, starch and lactose.

Figures (a & b in 4, 5 and 6) show that when the culture media were not supplemented with nitrogen sources, antifungal yields dropped to the minimum values. Potassium nitrate was the best nitrogen source for antifungal compounds production followed by peptone.

The production of antifungal compounds increased gradually from 25°C till the optimum temperature at 37°C and then decrease with increasing temperature for the selected bacterial species (Figs. a & b in 7, 8 and 9).

The pH 7 was the best value for antifungal compounds production by the three selected bacterial species (Figs. a & b in 10, 11 and 12). Below and/or above such pH value, the antifungal production decreased to various levels.
The antifungal production from the three selected bacterial species was higher under shaking condition rather than static (Table 2).

Discussion: Detection and identification members of bacteria are of value because they provide a source of antibiotics. Towards the goal of identifying additional novel antibiotics, a total of one hundred bacterial isolates were recovered from three soil samples collected from Egypt. They were tested for antagonistic activity against some human-and plant-pathogenic fungi. 20% isolates showed antagonistic activities of variable degrees against all or some of six tested human-and plant-pathogenic fungi. The obtained results was higher than those reported by Magnusson et al.[16] who found that 10% of the 1200 isolates of lactic acid bacteria isolated from different environments showed inhibitory activity against fungi. Out of 20%, the most active three isolates were identified as Tsukamurella inochonensis, Corynebacterium nitroliophilus and Cellulosimicrobium cellulans. The inhibitory effect of the three tested bacterial species may be primary due to the diffusion of an antibiotic into the medium which is in accordance with that reported by Yuan and Crawford[23].

Many factors play an important role in the process of antifungal production and consequently affect the antagonistic activity of the bacterial species. Carbon compounds constitute the major requirement for growth as they enter in different metabolic process resulting in the production of primary and secondary metabolites including antifungal compounds. Investigating the effect of various carbon sources on antifungal production by the three selected isolates, it was found that glucose was the best carbon source for antifungal production. Similar results were obtained by many workers[22,4,6].

Nitrogen is the second major requirement for the growth and the different metabolic activities of microorganisms as it enters in the synthesis of cell structural and functional proteins. It is well known that changes in the kind of nitrogen source greatly influence antibiotic production. Nitrogenous compounds may influence the biosynthesis of antibiotics directly at the level of secondary metabolism, either through their availability as substrates for antibiotic biosynthesis or through modulation of biosynthesis/activity/stability of the enzymes[21]. It was found that potassium nitrate is the best nitrogen source for antifungal production for the selected bacterial isolates. Similar results were obtained by Jonsbhu et al.[14]. This result may be due to that growth on nitrate as the only nitrogen source revealed to exponential growth phase, and the replicatory growth seems to remain constant during the second growth phase. This finding agrees with that reported by Heydorn et al.[10].

Concerning the effect of different temperatures on antifungal production by the three selected bacterial species, it was found that the optimum temperature for antifungal production was 37°C. Similar results were obtained by Celia et al.[3]. The above result may be due to that moderate temperatures are suitable for growth and consequently for the activity of enzymes responsible for secondary metabolite biosynthesis. This finding runs parallel with that reported by Nadkarni et al.[7].

Elibol[9] mentioned that pH is a critical factor for microbial growth and metabolic biosynthesis which affected antibiotic production. It was found that the optimum pH-value for antifungal production by the three selected bacterial species was 7.0. Similar results were obtained by EL-Abyad et al.[5] and Gong et al.[9].

The effect of shaking on the antifungal production for the three selected bacterial species incubated in static and shaking states revealed that the antifungal production was higher under shaking condition (150 rpm). Similar conclusion was obtained by Patelvo et al.[18]. The above result may be due to that under static conditions, the O2 is insufficient. This reflects the importance of O2 for both growth and consequently the antifungal production[9]. The antagonistic profiles of these active strains encourage the authors to isolate and identify the antifungal compounds produced by these active strains[9].

REFERENCES


