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Investigation on Antibacterial Activity of Some *Antibacterial*

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ABSTRACT

Bacteria is one of limitation factors in agricultural crops. According to their adaptation to various environmental conditions, their destructive effects in harvesting and emerging resistant races to common bactericides in their populations, which apply new controlling measures, are indispensable. In this case, using secondary metabolites of fungi, like *Penicillium* with antibacterial effect could be promising. In this survey 6 fungal species such as *Penicillium chrysogenum, P. citrinum, P. pinophilum, P. crustosum, P. cammune* and *P. aurantiogriseum* with two genres of plant pathogenic bacteria, *Rathayibacter iranicus* and *Pseudomonas syringae* were used. In this study four antibacterial assays were used: cultivation at broth media, dual culture, antibiotic and four-point cultivation assay, and that all of the experiments were performed with three repetitions. Results of this investigation indicate that these six species of *penicillium* have antibacterial effect on *Rathayibacter* and they can be controlled it but no antibacterial effect was observed in *Pseudomonas*, and bacterium were able to control the fungus additionally.
KEYWORDS: Antibacterial, Investigation, Activity

INTRODUCTION

Some aspects of soil biological activities belong to fungi. Soil inhabiting fungi is from different taxonomic groups (25). *Aspergillus* and *Penicillium* are the most frequent soil inhabitant fungi and could be found in all areas with dry to moist soils (1). In soil biology, biological activity could be assessed by the soil inhabiting organisms, so generalization of the results to the special soil inhabiting groups like Actinomycetes is not possible and each of these organisms play roles in all biological processes. In order to achieve this goal, isolation, identification and studying the soil biological characteristics are indispensable (21). Soil biology and the biochemistry of enzymes involved in soil were studied extensively which are used as index to measure the soil biological action (18). Probably all of the studied microorganisms have enzymatic activity which cause such conclusion. Isolation and purification of the secondary metabolites and their evaluation in soil are so difficult, hence vitro studying of these metabolites with the use of pure cultures, seems to be simple and reliable. Biologists are more interested in antibiotics as growth inhibitors of microorganisms among the secondary metabolites. *Aspergillus* and *penicillium* are the main antibiotic producers which can be released in various medias, particularly in soil and can affect other microorganisms (25). Antibiotics are among the most prescriptive drugs around the world because of the emergence of resistant bacteria, and they have been encountered with serious concerns (23). Recent achievements in the development of new antibiotics, like chemical synthesis tools are perused but nowadays only a small number of new antibiotics are produced using medical industry(8). Other strategies are organic synthesis (4) changes in drug using nanotechnology (17) and deal with molecules with different mechanisms (20). Natural compounds were the most important antibiotic sources in the past (5). Production of antibiotics since the discovery of β-lactam antibiotic which had been produced by microorganisms (9) was followed by polychitins the second and third generation of β-lactams to the oxazolidinones and daptomycin (33) that are continued till now. In contrast to synthetic molecules with effective antimicrobial properties, natural compounds are still promising (28).And the new methods are described for discovering new drugs with biological resources (22).Researches on new strains of microorganisms were done but their antibacterial activity with valuable molecules in order to develop new antibiotics are not evaluated (35).Main sources of microorganisms were studied, due to the effect of environment to the microbial metabolism proposed, therefor microbial antibiotics were isolated form plant endophytes and organisms extensively.

Because of their major biological changes in soil habitats, soil microorganisms are extremely important in biochemical cycles. Qualitative and quantitative methods of soil inhabiting fungi evaluation are well described. Many fungal extracellular extracts with antimicrobial activity were found and majority of these compounds were isolated from the filamentous *Penicillium* fungus. Since the discovery of penicillin, fungi as producers of antibiotics and other secondary metabolites with biological activities are known. There is report of *P. brefeldianum* producing

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folic acid (Kurban*et al.*, 1981) which it's anti-viral, anti-fungal, antioxidant and antibiotic activity have been proven previously. Funiculosin compound with antibiotic activity is isolated from *P. funiculosum* (2) and also the SQ 30,957 is a new antibiotic produced by the mentioned fungus (33). Several compounds including penitremi A, B, C, D, E and F, brom, penitermi A, F and dehydro, penitermi D were isolated from *P. ochlochloron* but their antibacterial properties have not been investigated (30). According to these facts, *Penicillium* fungi secrete antibacterial antibiotics which can eliminate certain bacteria in the soil. In this study, we examined the antibacterial antibiotic production ability and bacterial growth prevention of *penicillium* species isolated from agricultural soils in Iran with stimulation of some isolates by adding bacterial colonies to their fungal media.

MATERIALS AND METHODS

6 species of *penicillium* were used in this test.
In order to evaluate the anti-microbial properties of samples, several anti-bacterial tests were used:

First assay
1- **Fungus preparation:** first fungi were cultured in PDA (Potato Dextrose Agar) then spore suspension was prepared and the concentration of spores was measured. For spore suspension preparation, fungi were cultured on PDA media and after fungal growth and spore production, 5 ml of sterile distilled water was added and placed on the stirrer for 2 minutes to mix the spores with water, then distilled water containing fungal spores poured into a sterile container. After this step, the number of spores was measured by a hemocytometer lam.
2-**Bacterium preparation:** bacteria were cultured in NAS (Nutrient Agar Sucrose) Media and after a week bacterial suspension with a certain concentration was prepared. Bacterial colony was added to the 1.5ml vials containing sterile distilled water then the concentration was adjusted with spectrophotometer.

First assay
For anti-bacterial test, potato dextrose broth (PDB) was prepared. Then these media were inoculated with bacterial and fungal spore suspensions. One control for each of bacteria and one for each of fungi were considered. Bacterial control is a medium without fungus and fungal control is a medium without bacterium. Samples were incubated at room temperature on a shaker with 120rpm. Tests were performed in a three iteration.
Second assay
In this test PDA medium prepared and inoculated with bacteria cultured, after the complete growth of bacteria, chloroform was used to kill them and then removed from the medium with cotton, then fungi were inoculated in the same media and after the 5 days plates was observed.
Third assay
In this test PDA media was prepared in 10cm petri dishes. Each petri dish was divided into two parts, then bacterium was cultured on one side and the fungus was cultured on the opposite side, in front of the fungus. This test is called "dual culture test".
Forth assay
PDA media was prepared in 10cm petri dishes and bacteria were cultured at 4 points around the petri dishes and the fungus were cultured at the center, then growth rate and shape of the colony were investigated.

RESULT AND DISCUSSION

Some species among the studied fungi indicated valuable metabolites production with industrial and commercial usage which is worth further studying. Bactria used in this study is plant pathogenic and fungi were collected from agricultural soils of different provinces with different climates. Results of this research indicated anti-bacterial activity of fungi against *Rathayibacter* while no anti-bacterial activity was observed against *Pseudomonas*. *Penicillium aurantiogriseum, P. chrysogenum* and *P. cammune* showed positive antibiotic responses against *Rathayibacter* but negative against *Pseudomonas* while *P. citrinum* showed positive results against both bacteria, and fungal growth was limited in the medium. Also dual culture tests showed interesting results, all tested fungi grew easily when cultured with *Rathayibacter* and it was a failure to prevent fungal growth when compared with *pseudomonas* which can prevent fungal growth and inhibition zone develops around the fungus. Four-point cultivation assay had similar results in contrast with dual culture test. In this assay fungi grow easily against *Rathayibacter* but *Pseudomonas* bacteria could prevent the growth of fungi. According to the results, these fungi have the ability to control some bacteria in soil and the presence of these fungi in soil is useful for reducing crop damages.

Natural compounds with pharmaceutical uses played an important role to treat human diseases and that microbial environments are important sources of natural active agents (29). Many compounds that are currently used in wide ranges are obtained by microbial fermentations or chemical modifications of biological compounds (12). In this context, fermentation is an efficient process for secondary environment constructive compounds, availability of food and *etc*. 3-7-dimethyl-8-hydroxyl-6-metoxysocroman was isolated from the mycelium of *P. corylophilum*, which had been incubated at 22°C for 12 days in a medium containing grinded wheat (7). Epoxyagroclavin1 alkaloid was identified from culturing *P. corylophilum* strains in malt extract media (15) and 8-0-methyl sclererotiorinamin with antimicrobial properties in *P. multicolor* was detected (Nam *et al.*, 2000). Two active compounds, 8-0-methylaverafin and 1-8-0-dimethylaverantin were isolated from *P. chrysogenum* as new antifungal agents (24). Two antibiotics with new antifungal properties were isolated from *Aspergillus fumigates* isolates (26). In an effort to find cell cycle inhibitors with microbial origins, diketopiperazin was isolated from culture fermentation of *A. fumigates* fungus (6).Mentioned substances may play a role in bacterial control of *Penicillium* species tested in our experiments, which require further investigations about these issues in the future. metabolites production which could not be extracted from plants and animals and even could not be produced by genetic manipulations (10). According to Gaden-Junior(2000) metabolites production by microorganisms is effected by

Table 1. Bacterial and fungal cultures in broth media

	Pseudomonas	***Rathayibacter***
P. chrysogenum	- - -	+++
P. citrinum	- - -	+++
P. pinophilum	- - -	+++
P. cammune	- - -	+++
P. aurantiogriseum	- - -	+++
P. crustosum	- - -	+++

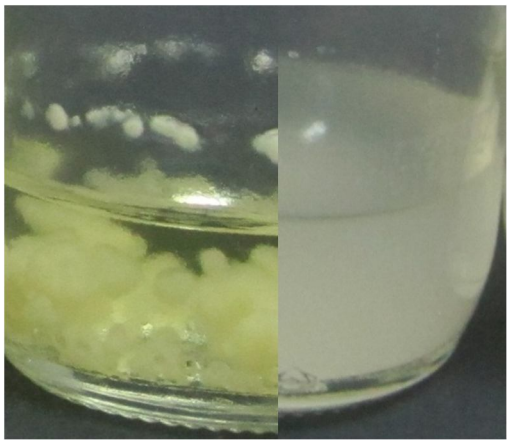

Fig. 1. negative and Positive results of broth media cultivation test, from right to left.

General conclusion

Part of the events that occur in the soil could be simulated in laboratory conditions. In this study results indicate that several species of *Penicillium* have the ability to control some plant pathogenic bacteria. Subsequent studies regarding beneficial and harmful metabolites of microorganisms could be promising.

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