

## Evaluation of Antibacterial Activity of Different *Penicillia* Isolated from Agricultural Soils

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### Abstract

In an experimental study, anti-bacterial activities of more than eighty soil inhabitant *Penicillium* isolates were evaluated. Isolates were cultured, simultaneously with bacteria: *Pseudomonas syringae*, *P. viridiflava*, *Xanthomonas translucens*, *Agrobacterium tumefascines*, *Rathayibacter iranicus*, and one isolate of *E. coli* in PDB and the results were evaluated after one week. All tested isolates, had anti-bacterial activity against one or more bacterial strains tested. It seems that, most of the examined *Penicillia*, produce one or more antibacterial substances and are able to prevent growth and proliferation of bacteria in their habitat.

**Keywords:** Antibacterial activity; Soil fungi; *Penicilium*

### Introduction

Part of the soil biological activity is a result of terrestrial fungi. Soil fungi, include various groups and belong to different taxonomic groups [1]. In between, *Penicillium* and *Aspergillus* are among the most abundant fungal flora of soils of different regions and are found in almost all areas of dry and wet soils [2]. In concept of soil biology, evaluation of soil biological activity can be performed in general and in all living organisms in the soil. Hence, separation and generalization of results is not possible for specific groups such as fungi and bacteria or actinomycetes. Until, each share will be determined in biological process series. To achieve this purpose, isolation, identification and study of specific biological features of different groups of microorganisms seems necessary [3]. In most biochemistry and soil biology sources, soil enzyme activities have been studied which are used as indicators for measuring the biological activity of soils [4]. Presumably, all of microorganisms that have studied for enzymatic activity are shared in results. Isolation and purification of secondary metabolites from soil and their specific evaluation is difficult due to biological and physical conditions of soils. Hence, in-vitro studies on production of secondary metabolites seem simple and reliable process, using pure cultures of producing microorganisms. Between different bundles of secondary metabolites, biologists are especially interested in antibiotics as growth inhibitors of other microorganisms. Fungi are one of the main producers of antibiotic substances that in different environments, particularly soil, are able to produce and effect on other microorganisms [2]. In this research, we tried to investigate the ability of producing antibacterial substances, and preventing the growth of gram-positive and gram-negative bacteria, by different *Penicillium* species which are isolated from various regions of agricultural soils of Iran.

### Materials and Methods

#### Fungal isolates

Fungi used in this study, were isolated by culturing soil suspension in potato dextrose agar containing 0.01g streptomycin sulfate and 0.03g Rose bengal antibiotics. Then, spore suspension of pure culture of isolates was prepared in sterile distilled water and was used for inoculation of treatments.

#### Bacterial isolates

Isolates of the bacteria were prepared of living cultures from laboratory of bacteriology, Department of Plant Pathology at the University of Agricultural Sciences of Sari, and Biotechnology Laboratory at Biotechnology Research Institute of Mashhad University.

Isolates grown on NA medium and inoculum were prepared in sterile distilled water and were kept in the refrigerator.

#### Assessment of production of bacterial growth inhibitors

*Penicillium* obtained isolates, were cultured in 50 ml of potato dextrose agar medium (PDB) and were placed on shaker with 120 rpm speed [5]. Then 2 days later, were inoculated by bacterial prepared suspensions. Results were evaluated after one week rocking on shaker with observation of transparency or opacity in compared with control cultures.

### Results and Discussion

Fungi belonging to different groups, have been studied for production of some secondary metabolites in laboratory and natural conditions [6] that led to introduction of genus and species which are superior in studied characteristics [3]. Metabolites can enhance abundance and biological components of soils or some of them can be effective in reducing population of soil living organisms. Also, metabolites can have some values as well as commercial and industrial importanc [7,8].

Most of the bacterial isolates used in this study were gram negative and plant pathogenic. *Penicillium* isolates were obtained from

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agricultural soil of some provinces of Iran which had various climate and different geology.

*Pseudomonas syringae* is a rod shape, gram-negative bacterium with polar flagella. It is a plant pathogen which can infect a wide range of plant species. Being Gram-negative bacteria, most *Pseudomonas spp.* are naturally resistant to penicillin and the majority of related beta-lactam antibiotics, but a number are sensitive to Piperacillin, Imipenem, Ticarcillin, Tobramycin, or Ciprofloxacin. As results show, it is the more resistant bacterium to secreted secondary metabolite of all *Penicillium* isolates used in this study.

*Pseudomonas viridiflava* is a fluorescent, gram-negative, soil bacterium that is a minor pathogen on plants [9]. As you can see in table 1, all of the *Penicillium* isolates can control its growth.

*Agrobacterium tumefaciens* (updated scientific name: *Rhizobium radiobacter*) is the causal agent of crown gall disease (the formation of tumors) in over 140 species of dicot [10]. It is a rod shape, Gram negative soil bacterium. Among the *Penicillium* isolates, only E10 was unable to prevent its growth.

*Rathayibacter* is a genus of bacteria in order Actinomycetales which are gram-positive soil organisms. Gram positive bacteria are more sensitive to secondary metabolites which affect cell wall. A75, A80 and C22 didn't let this bacterium grow.

*E. coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms [11].

The results showed that most investigated fungi have the growth inhibitory activity against one or more inoculated bacteria. Some isolates did not let any inoculated bacterial grow, while some treatments had oscillations in the opacity of the medium, ultimately lead to transparency and to overcome bacterial growth which was accompanied by them.

Figure1 shows the positive and negative treatments.

The results of performed treatments with the soil sampling locations are shown in Table 1.

Overall, more than 480 culture crosses were done, of which nearly 80 percent of them showed a positive results.

*In vitro* culture results indicate the kind of events that can occur in micro-ecological environment of fungus life in farm soil. Our results showed that *Penicillium* species which are existed in agricultural soil are able to control some important plant pathogenic bacteria species. Further investigations and isolations of produced antibacterial substances can be effective to strengthen or reduction some helpful or harmful soil microorganisms.

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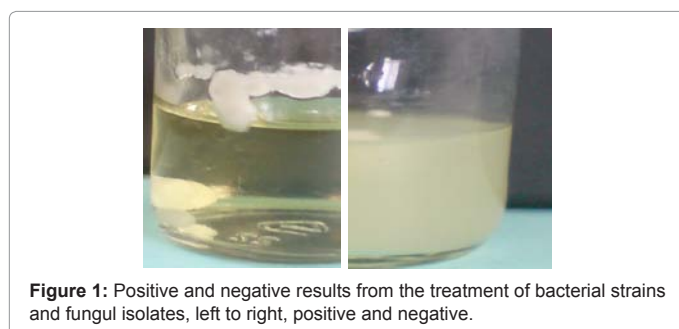


Figure 1: Positive and negative results from the treatment of bacterial strains and fungal isolates, left to right, positive and negative.

<i>Penicillium</i> isolates	Bacterial isolates					
	<i>P. syringae</i>	<i>P. viridiflava</i>	<i>X. translucens</i>	<i>A. tumefaciens</i>	<i>R. iranicus</i>	<i>E. coli</i>
A75	-	+	+	+	+	+
A80	-	+	-	+	+	-
C22	+	+	+	+	+	+
B254	-	+	-	+	-	+
E10	-	+	-	-	-	+

Table 1: Results of simultaneous culturing of bacteria and *Penicillium* isolates.