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# Induction and Enhancement of Antimicrobial Activity Produced by Fungal Isolates

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**ABSTRACT** Eight fungal isolates, which were isolated from soil samples, were used in the study. The isolates were identified as *Aspergillus* sp. (three isolates), *Trichoderma* sp. (two isolates) and *Penicillium* sp. (three isolates). All isolates were introduced to live cells of pathogenic bacteria, *Pseudomonas aeruginosa*. All antibiotic producing isolates showed enhanced antimicrobial activity towards *Bacillus subtilis*, and one of the non antibiotic producing isolates was induced to produce similar compound. The results suggested that the production of antimicrobial compounds produced by soil fungi can be induced or enhanced by the presence of pathogenic bacteria.

Keywords: Antimicrobial activity, induced activity, enhanced activity

## INTRODUCTION

For the past 50 years, antibiotics have revolutionised medicine by providing cures for life-threatening diseases. However, strains of pathogenic microbes have recently emerged that are virtually unresponsive to antibiotics. Such multi-drug resistance, arising mainly through antibiotic misuse, is now recognised as a global health problem. The situation is exacerbated by the fact that no novel antibiotics have been discovered for 20 years. Although many pre-existing antibiotics have been modified to yield new derivatives, pathogens have the potential to mutate to combat these antibiotics [1, 2]. Due to this, it is clear that new classes of antibiotics are urgently needed. New ways of screening for these compounds should be applied.

Few researchers have found out that certain strains of bacteria can be induced or enhanced to produce antibiotics [3, 4]. They appear to be doing so in response to chemical signals received from potential competitors strains which elicit in an antagonistic response.

Competition amongst microbes for space and nutrients in their natural environment is a powerful selective force which has led to the evolution of a variety of effective strategies for colonizing and growing on surfaces. These microorganisms can produce secondary metabolites which inhibit the settlement of potential competitors, and can antagonise other microbes.

The production of antimicrobial compounds by soil microorganisms is usually assayed under straightforward growth conditions and only strains which constitutively produce such compounds can be successfully screened. However, as the primary role of antimicrobial activity is to antagonise competitors, microbes may also produce antimicrobial compounds when they sense the presence of competing organisms [5].

Apart from antibiotic production significance, the knowledge about antagonism in a population of microorganism has also led to the searching for biological control agents especially in the field of agriculture [6, 7].

In this study, we have screened eight fungal isolates for the production of antimicrobial compounds and investigated the ability of live cells of pathogenic bacteria to elicit or enhance antibiotic production in these isolates.

## MATERIALS AND METHODS

### Isolation of soil fungi

Three soil samples were taken from Bukit Cherakah, Shah Alam. One gram of each sample was suspended in nine milliliter of

sterile distilled water. Ten fold serial dilutions were made. 0.1 ml from $10^{-5}$ tube was lawned on potato dextrose agar (PDA) containing chloramphenicol. The plates were incubated at room temperature for three days. Representative colonies were picked and isolated in a pure culture before determination of antimicrobial activity.

**Tester strain**

*Bacillus subtilis* was used in the antimicrobial assays. The bacterium was obtained from Universiti Teknologi MARA Microbiology Laboratory (UiTMcc) and was maintained on nutrient agar.

**Growth and culture condition**

Eight fungal isolates were chosen and designated as Asp1UiTMcc, Asp2UiTMcc, Asp3UiTMcc Tcd1UiTMcc, Tcd2UiTMcc, Pen1UiTMcc, Pen2UiTMcc and Pen3UiTMcc. All isolates were grown in potato dextrose broth (PDB) for 10 days at room temperature and agitated at 150 rpm. Their broth was used to determine the ability to produce antimicrobial compounds active against *Bacillus subtilis*. The strongest activity was determined after 8 days of fermentation (untreated).

In the respective fermentation broth, 100 ml of PDB of fungal isolates (10 spores/ ml) were introduced to one ml live cells of *Pseudomonas aeruginosa* (100 cells/ ml). The mix culture broth was incubated as before. The antimicrobial activity was assayed at 8 days fermentation (treated).

**Screening of antibiotic activity**

Antimicrobial activity was assayed using a paper disc assay [8] and 6 mm paper discs (Whatman) was used. Paper discs were saturated with culture supernatant fluid and placed onto nutrient agar seeded with tester strain, *Bacillus subtilis*. Plates were then incubated overnight at 37°C. Production of antimicrobial compounds was determined by measuring the diameter of inhibition zones.

## RESULTS AND DISCUSSION

The data presented in Figure 1 showed that the exposure to live cells of *Pseudomonas aeruginosa* induced and enhanced the antimicrobial activity of these fungal isolates but some are more enhanced than others.

The greatest enhanced activity was seen in *Tricoderma* sp isolate 1 (Tcd1UiTMcc) where the inhibition zone produced shown to increase to more than twice (from 8.5mm to 17mm) the inhibition zone when using first run fermentation broth. All other antibiotic producing isolates also showed enhancement of antimicrobial activity but not as much as Tcd1UiTMcc.

Induction of antimicrobial activity was seen in Pen3UiTMcc, which is an increase in inhibition zone of 6mm. Non antibiotic producing isolate, Asp3UiTMcc does not show anti *Bacillus subtilis* activity.

Table 1: Fungal isolates obtained from soil samples

| Isolates | Microscopic observations |
|---|---|
| Asp1UiTMcc | *Aspergillus* sp. |
| Asp2UiTMcc | *Aspergillus* sp. |
| Asp3UiTMcc | *Aspergillus* sp. |
| Tcd1UiTMcc | *Trichoderma* sp. |
| Tcd2UiTMcc | *Trichoderma* sp. |
| Pen1UiTMcc | *Penicillium* sp. |
| Pen2UiTMcc | *Penicillium* sp. |
| Pen3UiTMcc | *Penicillium* sp. |

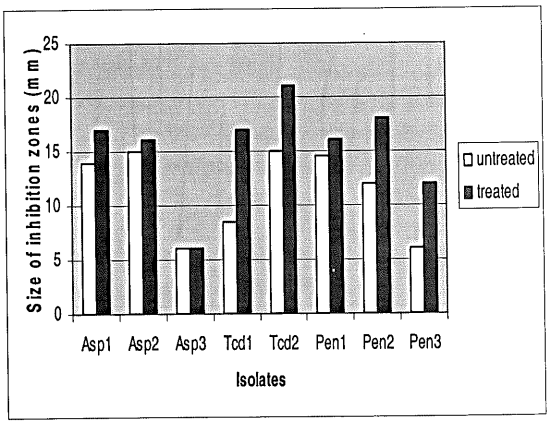


**Figure 1.** The effect of live cells of *Pseudomonas aeruginosa* on antibiotic production by eight fungal isolates.

Note: size of paper disc is 6 mm.

The above results demonstrated cross-species induction and enhancement of antimicrobial

activity by soil fungi in response to the presence of known pathogenic bacteria in the broth medium. It shows that the soil fungi are able to express new secondary metabolic functions in response to other species. These results appear to be the first example of cross-species induction of antibiotic synthesis in soil fungi.

In conclusion, the observed soil fungi can elicit antimicrobial responses in different species. It is interesting to speculate that this response represents a chemically induced defense response when the microbes are faced with potential competing organism in their natural environment. Further studies could be done using agar diffusion assay which is due to cationic compounds absorbed to the cellulose disc and not diffused into the medium.

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