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Original Article

Screening the Antibacterial Potential of *Paecilomyces fumosoroseus* against Some Pathogenic Bacteria

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ABSTRACT

Objective: The aim of the present investigation was to test the antibacterial activity of an entomopathogenic fungus *Paecilomyces fumosoroseus* against pathogenic bacterial strains.
Methods: Therefore to achieve the above objective the cell free culture filtrate (CFCF) of the entomopathogenic fungus *Paecilomyces fumosoroseus* was obtained after 7, 14 and 21 days of incubation. The activities of these CFCF were then screened for their antibacterial potential by using agar well diffusion assay against the pathogenic bacterial strains.
Results: The CFCF obtained after 21 days of incubation exhibited highest activity against majority of pathogenic bacteria. It gave maximum activity against *Escherichia coli* MTCC 1679. The activity was good against *Bacillus subtilis* MTCC 441 and *Salmonella typhi* MTCC 733 whereas, it was moderate against *Pseudomonas aeruginosa* MTCC 6204. The zones of inhibition obtained against these were 32mm, 18mm, 12mm and 10mm respectively. However the metabolite showed no activity against *Klebsiella pneumoniae* MTCC 4032.
Conclusion: Results of this research work reveal that the entomopathogenic fungal isolate *Paecilomyces fumosoroseus* produce some potential antibacterial compounds after 21 days of incubation which could be used for production of broad spectrum and ecofriendly antibiotics.

Keywords: Pathogenic bacteria, entomopathogenic fungi, secondary metabolites, antibacterial activity, Cell free Culture Filtrate (CFCF).

INTRODUCTION

The problem of antibiotic resistance is increasing worldwide among the most pathogenic bacterial strains.[1] This situation has attracted attention of researchers towards exploration of different natural resources for the development of novel and more potent antimicrobial agents which would ultimately minimize the possibility of further antimicrobial resistance.[2-4] Fungi are known to possess some unusual properties including antimicrobial properties against pathogenic bacteria, fungi and protozoan since many years.[5] Entomopathogenic fungi also have such potential. India is conferred with an abundant biodiversity of entomopathogens and benefits of these

natural and renewable sources are essential as antimicrobial products. Fungi are the most promising candidates for obtaining natural antimicrobial compounds for human use since they are similar to animals and might have common microbial enemies also.[6] Fungal bioactive compounds are therefore more beneficial for mankind as compared to any other natural ones.

Entomopathogenic fungi are classified as fungi that attack, spread and eventually kill their host insect. [7] *Paecilomyces fumosoroseus* is one of the most potential entomopathogenic fungus.[8] *Paecilomyces fumosoroseus* (Wize) Brown and Smith[9] show great insecticidal activity against various insect pest of which whiteflies and *Bemesiatabaci* are the most important ones.[10] They produce a disease called "Yellow Muscardiane".[11] Several more studies have also indicated that the entomopathogenic fungus, *Paecilomyces fumosoroseus* is highly valuable microbial insecticide as well as it is considered to be an efficient resource of active antibacterial compounds. Entomopathogenic fungi are successfully being used as biological control agents against variety of insect pests, but its antibacterial potential is still not much explored.

Some investigations have revealed that the entomopathogenic fungi usually produce toxic secondary metabolites which act as antimicrobial agents.[12] Compound like beauvericin has been isolated from *Paecilomyces fumosoroseus*[13] which has a strong antibacterial activity against several pathogenic bacteria.[14] Because of its broad spectrum antibacterial activity beauvericine is used to treat severe bacterial infections and non-food crop diseases.[15,16]

Therefore, this research work was undertaken with the aim of screening the antibacterial potential of *Paecilomyces fumosoroseus* against some important pathogenic bacteria.

MATERIALS AND METHODS

Entomopathogenic fungus and test pathogenic bacteria

Entomopathogenic fungal strain *Paecilomyces fumosoroseus* was kindly provided for this investigation from Fungal Biotechnology and Invertebrate Pathology Laboratory, Department of Biological Sciences, RDVV, Jabalpur, (M.P). Five pathogenic bacterial strains *Bacillus subtillis* MTCC 441, *Escherichia coli* MTCC 1679, *Salmonella typhi* MTCC 733, *Klebsiella pneumoniae* MTCC 4032 and *Pseudomonas aeruginosa* MTCC6204 were obtained from the Microbial Type Culture Collection Centre (MTCC), Chandigarh.

Media preparation and culture inoculation

25 ml of Potato Dextrose Agar medium was prepared and autoclaved at 121°C or 15 psi for 30 min. The pure culture of *Paecilomyces fumosoroseus* was then inoculated into sterilized plates containing potato dextrose agar medium under aseptic conditions. These plates were kept at 28±2°C temperature for 5 to 7days in incubator for growth.[17]

Production of secondary metabolite

The pure culture of *Paecilomyces fumosoroseus* was then inoculated in the Potato Dextrose Broth (PDB) medium in form of small discs separated with the help of cork borer from the plates. This fungal culture was grown in 50ml, 75ml and 100ml of PDB at the temperature of 28±2°C for 7, 14 and 21 days respectively. The cell free culture filtrate (CFCF) was obtained from the above three flasks by filtering the culture broth through Wattman filter paper No.1 after the proper incubation period is over.. The CFCF obtained after 7, 14 and 21 days of incubation were treated as the secondary metabolite and further screened for the antibacterial potential.[8]

Screening of secondary metabolite for antibacterial activity

The metabolites obtained after 7, 14 and 21 days of incubation were screened for their antibacterial activity against the test pathogenic bacterial strains, viz. *Escherichia coli* MTCC 1679, *Salmonella typhi* MTCC 733, *Klebsiella pneumoniae* MTCC 4032, *Pseudomonas aeruginosa* MTCC 6204 and *Bacillus subtilis* MTCC 441. This was done by using agar well diffusion assay.

Agar well diffusion assay

Agar plates were seeded with 20-30 µl of bacterial culture and lawn was prepared by spread plate method and allowed to dry for 30 minutes. With the help of sterile cork borer wells of 4 mm diameter were made in the plates containing bacterial culture under aseptic condition. 50-80 µl of metabolite was dropped in the prepared wells and plates were kept in bacteriological incubator at 37°C for 24 hours. Finally after incubation the zones of inhibition obtained in plates were measured.[18]

RESULTS AND DISCUSSION

Maintenance of pure cultures of Entomopathogenic fungus and test pathogenic bacteria

The pure cultures of entomo-pathogenic fungus *Paecilomyces fumosoroseus* (Fig. 1.1) and test pathogenic bacterial strains *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 1679, *Salmonella typhi* MTCC 733, *Klebsiella pneumoniae* MTCC 4032, *Pseudomonas aeruginosa* MTCC 6204 (Figure 1.2 a-e) were maintained by regular sub culturing on nutrient agar media and stored at 4ºC temperature in refrigerator. Likewise the pure culture of the fungus *Paecilomyces fumosoroseus* was maintained on potato dextrose agar slants at 4ºC.[19]

Production of Secondary Metabolite

Production of antibacterial meta-bolites by *Paecilomyces fumosoroseus* was found to be best in the potato dextrose broth (PDB). The medium inoculated with the test fungus was incubated at 28°C and pH 6 was found to be optimum for the secretion of metabolites by the fungus (Fig 2.1a). The fungal biomass was then separated by filtering through Wattman Filter paper No. 1 and the filtrate thus obtained also known as cell free culture filtrate (CFCF) was screened for antibacterial activity (Fig 2.1b). Similarly, fungal metabolites of several specific genera: *Paecilomyces*, *Polyporus*, *Isaria* and *Beauveria*, were isolated by Luangsa *et al.*[20] This metabolite showed significant bioactive properties.

Screening of Secondary Metabolite for Antibacterial Activity

The activity of metabolites produced by *Paecilomyces fumosoroseus* after 7, 14 and 21 days of incubation were screened for their antibacterial potential against following pathogenic bacterial strains; *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 1679, *Salmonella typhi* MTCC 733, *Klebsiella pneumoniae* MTCC 4032 and *Pseudomonas aeruginosa* MTCC 6204. This was done *via* agar well diffusion assay and it was found that the metabolite obtained after 21 days exhibited maximum activity against all the test bacteria followed by 14th and 7th day metabolite (Fig. 3.1). According to this investigation the metabolite of 21st day showed highest activity against *Escherichia coli* MTCC 1679 by giving an inhibition zone of 32 mm followed by *Bacillus subtilis* MTCC 441 and *Salmonella typhi* MTCC 733 the zones obtained against them were 18 mm and 12 mm respectively. On the other hand the metabolite exhibited moderate activity against *Pseudomonas aeruginosa* MTCC 6204 with an inhibition zone of 10 mm whereas it showed no activity against

Klebsiella pneumoniae MTCC 4032 (Table 1) (Fig 3.2 a-d). Likewise the broad spectrum activity of several strains of fungi was tested against a wide range of Gram positive and Gram negative bacteria by Mekawey.[1] Out of these some of them were found to be potential source of thermo stable antibiotics. In the same way Luangsa-ard *et al.,* studied the production of bioactive compound beauvericine from several specific fungal genera: *Beauveria*, *Paecilomyces*, *Polyporus*, *Isaria* and *Fusarium* and checked their potential.[21]

CONCLUSION

The increasing number of antibiotic resistant bacterial strains has urged upon the need to search for new antibacterial drugs from natural sources. Hence, the present research work was undertaken to reveal the antibacterial potential of the entomopathogenic fungal strain *Paecilomyces fumosoroseus*. This strain was found to be active against four pathogenic bacteria out of the five used as test organisms. The activity of *Paecilomyces fumosoroseus* was maximum against *Escherichia coli* MTCC1679 followed by *Bacillus subtilis* MTCC 441, *Salmonella typhi* MTCC 733 and *Pseudomonas aeruginosa* MTCC 6204. Whereas it showed no activity against *Klebsiella pneumoniae* MTCC 4032. Thus, on the basis of this investigation it could be concluded that *Paecilomyces fumosoroseus* is an important source of antibacterial compounds which could be developed as antibiotics acting against a wide range of pathogenic bacteria. The specific antibacterial compound present in the fungal extract would be purified in future and investigated further for characterization spectrum.

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Funding Source Declaration

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Conflict of Interest

The authors have no potential conflict of interest regarding the publication of the said manuscript.

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Table 1. Screening of secondary metabolite for antibacterial activity (n).

Incubation Period	Antibacterial activity against test organisms (n= diameter of zone of Inhibition in mm)				
	***Bacillus subtilis* MTCC 441**	***Escherichia coli* MTCC 1679**	***Salmonella typhi* MTCC 733**	***Klebsiella pneumoniae* MTCC 4032**	***Pseudomonas aeruginosa* MTCC 6204**
7 Days	2	5	1	_	2
14 Days	7	10	5	_	4
21 Days	18	32	12	_	10

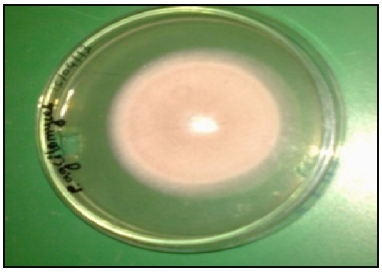


Fig. 1.1: Pure culture of *Paecilomyces fumosoroseus*

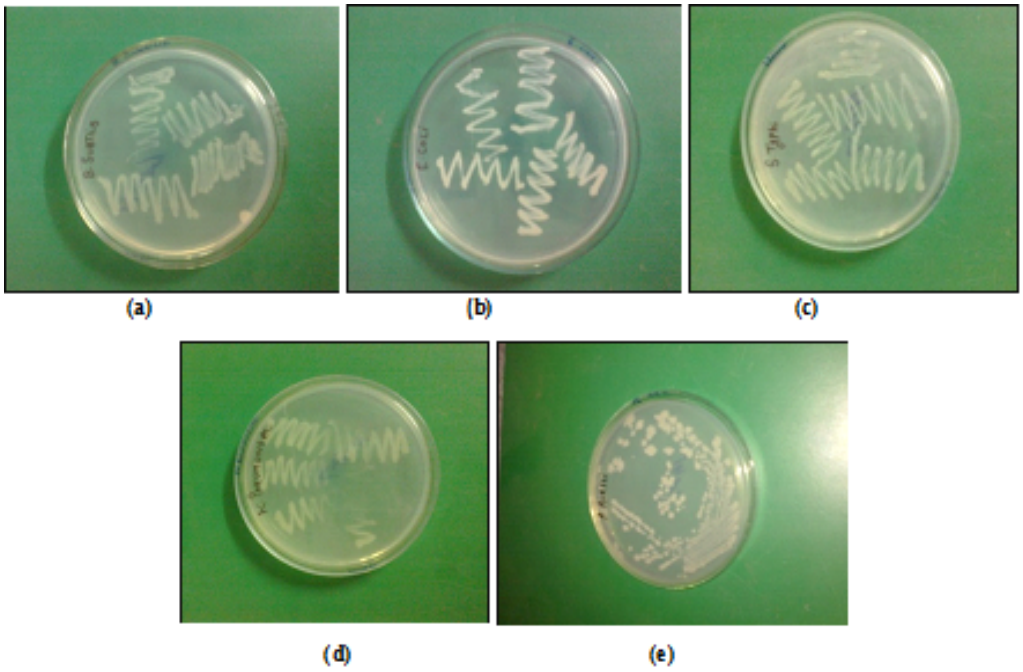
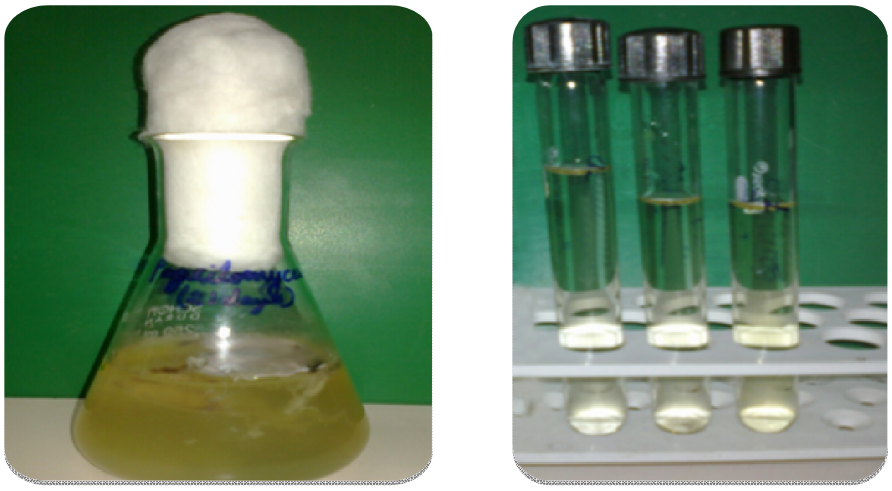


Fig. 1.2: Pure cultures of test pathogenic bacteria: a) *Bacillus subtilli* MTCC 441, b) *Escherichia coli* MTCC1679, c) *Salmonella typhi* MTCC733, d) *Klebsiella pneumoniae* MTCC4032, e) *Pseudomonas aeruginosa* MTCC6204.



2.1a **2.1b**

Fig. 2.1a: 21 days old PDB broth inoculated with *Paecilomyces fumosoroseus*,
Fig. 2.1b: Cell free culture filtrate (CFCF).

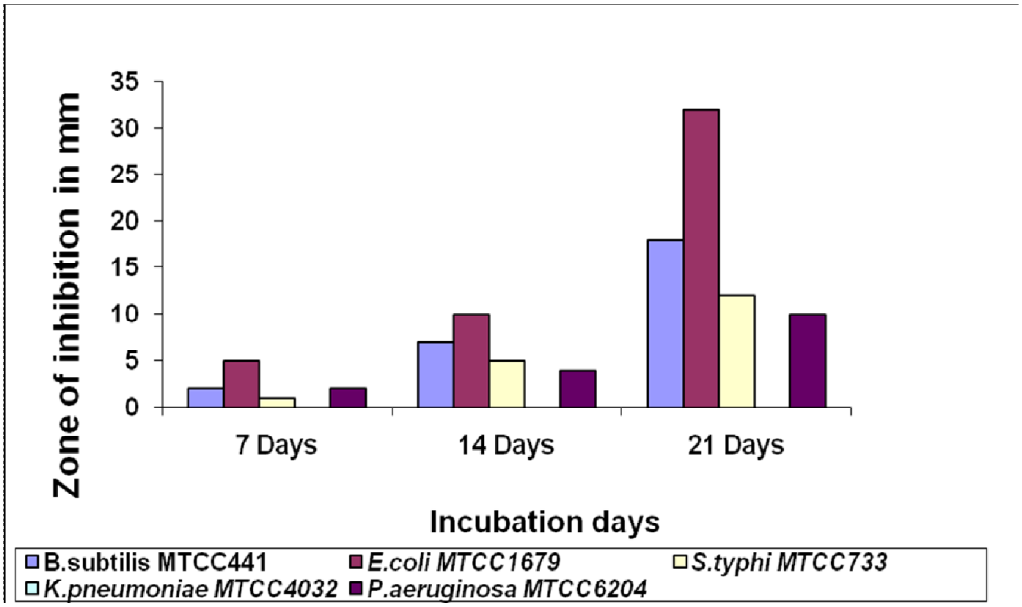


Fig. 3.1: Graphical representation of antibacterial activities of the secondary metabolite of *Paecilomyces fumosoroseus* against test bacterial strains after 7th, 14th and 21st day of incubation.

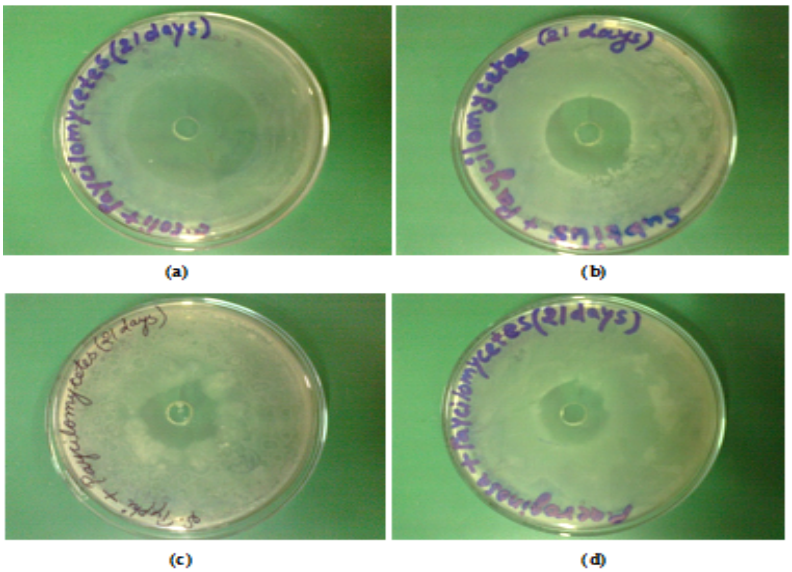


Fig. 3.2: Antibacterial activity of secondary metabolite of *Paecilomyces fumosoroseus* against: a) *Escherichia coli* MTCC 1679, b) *Bacillus subtilis* MTCC 441, c) *Salmonella typhi* MTCC 733, d) *Pseudomonas aeruginosa* MTCC 6204.