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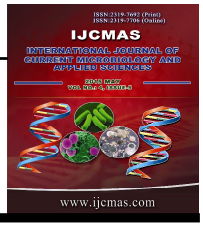

Original Research Article

Antimicrobial Activity of Endopytic *Penicillium sp.* and *Fusarium moniliforme* against MDR Human Pathogens

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A B S T R A C T

Plants are used as a medicine from ancient time in the treatment of many infections and microbial endophyte is a diverse and untapped source of biological diversity with an enormous bioactive potential. In the present study, the antimicrobial activity of the endophytic fungi Penicillium sp. and *Fusarium moniliforme* isolated from medicinal plant *Colocasia esculenta* was evaluated. These endophytic fungi were isolated from the leaves of the plant and the extracts were obtained using different solvents like Hexane, Ethyl acetate, Chloroform and water. The antimicrobial activity of fungal culture extracts was checked against six multi drug resistant human pathogens by standard protocol of agar well diffusion method and assessed by measuring the zone of inhibition. The results obtained from this study forms a base for further investigations on endophytic fungi of medicinal plants and their antimicrobial potential.

Keywords

Endophytes, Antimicrobial activity, Multi drug resistance, *Colocasia esculenta*

Introduction

Natural products have remained the most important resource for the discovery of new and potential drug molecules (Strobel and Daisy, 2003). There is a growing interest in endophytes, their origin, biodiversity, endophyte-host interaction, their role in ecology and the characterization of their secondary metabolites (Arnold, 2007; Saikkonen *et al.*, 2004). However, only a handful of plants, mainly grass species, have been completely studied in relation to their endophytic biology (Strobel *et al.*, 2004). Microbial endophyte is a diverse and untapped source of biological diversity with an enormous bioactive potential. The range of bioactive compounds produced by endophytes is very diverse.

Endophytes synthesizes a variety of secondary metabolites which are not directly involved in the metabolism of the micro-organisms but play a role in the fitness and survival of themselves and their host (Schulz *et al.*, 2002; Tan and Zou, 2001). These include alkaloids, flavonoids, phenols, steroids, terpenoids, quinones, isoquinones etc. It is believed that medicinal plants and their endophytic flora produce similar

pharmaceutical products (Rezwan Khan *et al.* 2010). Since, the discovery of endophytes by Darnel, Germany in 1904 (Tan and Zou, 2001), various investigations have been done on endophytes in different ways which is usually dependent on the prospective from which the endophytes were being isolated and subsequently examined. The use of endophytic fungus for the production of pharmacologically active metabolites has been on rise (Knight *et al.,* 2003). Research on endophytes reveals that, they are a huge source of medicinal compounds.

Colocasia esculenta is used as a medicinal plant in the treatment of skin diseases and also a well known vegetable. Literature revealed that less study is available on the endophytes associated with *C. esculenta*. Studies on the endophytic fungi associated with *C. esculenta* will be of great importance to understand its medicinal properties. The present study aims at the isolation and screening of endophytic fungi from *C. esculenta* and their antimicrobial activity against multi- drug resistant human pathogens.

Material and Methods

Isolation of Endopytic fungi

Healthy leaves of *C. esculenta* were collected, washed thoroughly under running tap water and were cut into 5to 6 mm diameter and 1 cm in length segments. The leaf segments were then subjected to surface sterilization by method given by Petrini (1986). Sterilized segments were placed on water agar medium supplemented with antibiotic chloramphenicol (50µg/ml). Plates were incubated at 27°C and observed for the growth. The hypal tips were transferred to fresh Potato Dextrose Agar plates to obtain pure culture. Sporulating structures were considered as diagnostic features for the morphological identification of endophytic fungi. The endophytes were also identified by ITS rDNA sequence analysis.

Antimicrobial activity

The fungal culture extract in liquid growth media was obtained by the method described by Chaoudhary *et al.* (2004). The culture media and mycelia were separated by filtration. The filtrate was extracted with different solvents like Ethyl acetate, Hexane, Chloroform and water and the endopyte culture extract was obtained by evaporating organic solvents under reduced pressure. The antimicrobial activity of fungal culture extracts was tested against multi drug resistant pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acenitobacter baumanii* and *Salmonella typhi* (Table 1).

30µl of endophytic culture extract was added to each well. DMSO was used as a negative control while chloramphenicol was used as a positive control. Plates were incubated at 37°C overnight and the results were recorded as zone of inhibition (in mm).

Results and Discussion

The antibiotic resistance pattern of test organism is depicted in table 1.

The endophytic fungi isolated from leaves of *Colocasia esculenta* were identified as *Penicillium sp.* and *Fusarium moniliforme.* The antimicrobial activity against multidrug resistant pathogens is detailed in table 2 and 3.

Various investigators reported endophytic microbes from various plants existing in different ecosystems.

Table.1 Antibiogram of test multidrug resistant pathogens

Antibiotics	***E. coli***	***S. aureus***	***P. aeruginosa***	***A. baumanii***	***K. pneumoniae***	***S. typhi***
Erythromycin	S	S	S	R	S	R
Ampicillin	S	R	R	R	R	S
Colistin	R	R	R	R	R	S
Vancomycin	S	S	S	S	S	S
Trimethoprim	R	S	R	R	R	R
Streptomycin	S	S	S	S	S	S
Tetracycline	R	S	R	S	S	R
Gentamycin	R	S	S	R	S	S
Chloramphenicol	S	R	S	R	R	R
Nalidixic acid	R	R	S	R	R	S
Norfloxacin	S	S	R	S	R	R
Kanamycin	R	R	R	R	S	R

Table.2 Antimicrobial activity of fungal culture extract of *Penicillium* sp against drug resistant pathogens

Sr. No	**Name of Organisms**	**Zone of inhibition(mm)**				
		Ethyl acetate	**Hexane**	**Chloroform**	**water**	**Cloramphenicol (+ve control)**
1	*E. coli*	21	15	16	15	22
2	*S. aureus*	19	18	15	13	21
3	*P. aeruginosa*	20	22	17	17	24
4	*A. baumanii*	21	20	19	18	23
5	*S. typhi*	19	18	15	14	20
6	*K. pneumoniae*	20	19	19	15	23

Table.3 Antimicrobial Activity of fungal culture extract of *Fusarium moniliforme* against drug resistant pathogens

Sr. No	**Name of Organisms**	**Zone of inhibition(mm)**				
		Ethyl acetate	**Hexane**	**Chloroform**	**water**	**Cloramphenicol (+ve control)**
1	*E. coli*	20	17	17	16	22
2	*S. aureus*	17	15	16	14	21
3	*P. aeruginosa*	22	20	21	18	24
4	*A. baumanii*	22	21	20	19	23
5	*S. typhi*	18	16	15	13	20
6	*K. pneumoniae*	20	17	18	16	23

Only few plant species have been completely studied for their relative endophytic biology. Consequently the opportunity to find new and interesting microorganisms among myriads of plants in different settings and ecosystems is great (Strobel and Daisy, 2003).

Since almost all plant species carries at least one or more endopytic microbe within it, research on endophytes displaying various bio activities could be a potent source of various metabolically active compounds against drug resistant pathogens. Both the endopytic fungi isolated from the leaf of *C. esculenta* showed almost same antimicrobial activity against multi drug resistant pathogens, which may be because of their presence in the same host. Further study on the bioactive compounds from endophytic fungi may help to isolate some potential bioactive compound which can act as a potential source of new antimicrobial agent.

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