Isolation of Endophytic Microorganisms As A Source of Novel secondary Metabolite Producers Against Tuberculosis

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Abstract- *In the present research study, isolation of endophytic microorganisms were carried out from Fresh body parts of Azadirachta indica and Parthenium hysterophorus plants collected from botanical garden of SMM murgud (M.H).Total 10 endophytic microorganisms were isolated and named them as R1, R2upto R10. Among thisIsolates 6 was Bacterial species and 4 was fungal species. From this 10 isolates, R2, R3, R5 and R10 showed highest antimycobacterial, antifungal and antibacterial activity which was again selected for further study. From present research study it can be concluded that, endophytic microorganisms are able to produce secondary metabolites and that can be used indifferent clinical applications.*

Keywords- Endophytic microorganisms, Antifungal and Antibacterial activity, Secondary metabolites.

I. INTRODUCTION

The need for discovery of new and useful drugs to provide effective treatment and relief in all aspects of humankind is increasing due to arise of many drug resistance bacteria, the appearance of life-threading viruses, fungal infection, environmental degradation and loss of biodiversity (Strobel & Daisy, 2003).An endophyte is an organism which lives inside a plant. This is contrasted to epiphyte, which refer to the organism on the outside of the plant.while Wilson (1995) considered endophytes as any fungus or bacterium found inside plant tissues.

Endophyte that occure intercellularly within the leaves,stems and reproductive organs of grasses have impressive effects on the physiology, ecology and reproductive biology of its host plant.They often protect their host plants against a wide range of insect and mammalian herbivores, through the production of toxic alkaloids.Endophytes are the chemical synthesizers inside plants. (Owen & Hundley,2004). Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been proved useful for novel drug discovery.According to Demain (1981),antibiotics compound is low-molecular-weight organic natural products made by microorganisms that are active at low concentration against other microorganisms, are the most bioactive natural products isolated from endophytes.In the present research, isolation and antimicrobial activity of endophyticbacteria from different body parts of Azadirachta indica is performed.

II. MATERIALS AND METHODS

Collection of samples

Fresh body parts of Azadirachta indica and Parthenium hysterophorus was collected from the botanical garden of SMM murgud (M.H). Samples were immediately brought to the laboratory and used within 24 hrs for the isolation of endophytic microorganisms.

Surface sterilization of samples

The leaves and roots were thoroughly washed in running tap water and immersed in 70% ethanol for 10 seconds, followed by 4% sodium hypochlorite for 1 minute and then rinsed in sterile distilled water for 1 minute; the excess moisture was blotted with a sterile filter paper. sterilized Samples were excised with sterilized scalpel and forceps under aseptic condition and dried for further use.

Sterility check

To confirm that sterility of sterilized Samples 0.1 ml of the sterile distilled water that was used in the final rinse of surface sterilization process were spreaded on sterilized NA media plates and incubated at 37^0C for 72 hrs.

Preparation and sterlization of media

Nutrient agar media were prepared by the addition of nutrient broth and agar agar powder in distilled water.

Hotplate was used for proper mixing of media and autoclaved at 121^{0}C for 15 mints. at 15lbs.

Preparation of sterilized plates

The autoclaved media was poured off into sterilized Petri plates and kept for solidification. After solidification plates were used for isolation of endophytic microorganisms.

Isolation of endophytic microorganisms

Sterilized Samples were inoculated on NA media plates. These plates were incubated at 37^{0}C for 72hrs.The grown cultures were maintained by sub culturing periodically and preserved at refrigerator.

Antibacterial activity of isolates

Antibacterialactivity of Endophytic isolates were screened against *Bacillussubtilus, Escherichia coli, pseudomonas auerogenosa, staphylococci spp and Mycobacteria tuberculosis* by agar well diffusion method.

Antifungal activity of isolates

Antifungal activity of Endophytic isolates were screened against Fusarium species and Trichoderma species by agar well diffusion method.

III. RESULTS

FigureI:-Collection of samples (a) Azadirachta indica (b) Parthenium hysterophorus

FigureII:-Isolation of Endophytic micro organisms

collection of Samples and isolation of endophytic microorganisms

From the Fresh body parts of Azadirachtaindica and Partheniumhysterophorus total 10 endophytic microorganisms was isolated shown in figure I and II. Among them 6 was bacterial species and 4 was fungal species and they were named as R1, R2.....R10.

Antimicrobial Activity of isolates

Isolates Endophytic microorganisms showed antibacterial activity and antifungal activity. R2, R3, R5 and R10showed highest antibacterial and antifungal activity and shown in Table I.

Table I antibacterial,antifungal and antimycobacterial activity of endophytic isolates.

Sr.No.	Isolates	Antibacterial activity (mm)	Antifungal activity (mm)	Antimycobacterial activity (mm)
1	R1	8	-	-
2	R2	16	28	4
3	R3	14	24	6
4	R4	7	13	-
5	R5	12	22	-
6	R6	6	9	8
7	R7	4	10	-
8	R8	7	-	-
9	R9	8	-	-
10	R10	10	22	7

IV. DISCUSSION

Microbial endophytes from medical plants are reported as source of original secondary metabolites (Leiter et al1950).This neglected group of organism is important component of biodiversity.They play vital physiological and ecological role in their life, by increasing the adaptability of their host (Kawada et al., 2010). These microorganisms are found to be affect by change in climate and environmental conditions. Tropical and temperate endophytes, found to be different with respect to metabolite production ability (Arai et al., 1973).

During course of investigation, we found that endophytes are tissue specific and affected by seasonal variations. Statistically it is proved that in rainy season endophytes are more in number than winter and summer. This finding also collate with work done by earlier researchers (Song et al., 2004).

Endophytes microbes are prolific producers of antimicrobial metabolites (kusari et al., 2008). We accept this statement as about 50% of our isolated showed potent antimicrobial activity against test organisms. Earlier, it was demonstrated that crude extracts from culture broth of endophytes displayed excellent antibiological activity (isaka et al., 2009). Our results are in good agreement when concerned with antimicrobial activity as stated by others.All endophytes tested confirmed robust antimicrobial activity against test microbes (Bagtell et al.,2004).

V. CONCLUSION

Plants containendophytic micro organisms and they are able to produce secondary metabolites and that are effective against above mentioned pathogens therefore they are useful in future clinical applications.

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