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Studies on endophytic bacteria isolated from grass against dysentry causing *shigella* species

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Abstract
As the bacterial resistance is increasing to drugs in various diseases, new areas for drugs production are being explored by researchers. From this one of the area is Bacterial endophytes. In the present research, we isolated 16 endophytic bacterial species from Grass collected from different regions of Pune and screened for the presence of antimicrobial compounds against *Shigella* species by disc diffusion method. From 16 endophytic bacterial species RB10 showed highest antimicrobial activity against *Shigella* species. These RB10 endophytic bacterial species was further selected for Production of secondary metabolites by submerged fermentation method.

Keywords: endophytic bacteria, secondary metabolites, antibacterial activity

Introduction
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. 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).
Endophyte that occur intracellularly 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 defence 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, production and partial purification of secondary metabolites from endophytic bacteria from different body parts of grass is performed.

Materials and Methods
Collection of samples
Fresh body parts of grass was collected from the different regions of Pune (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 sterilization of media
Nutrient agar media were prepared by the addition of nutrient broth and agar agar powder in distilled water. Hot plate was used for proper mixing of media and autoclaved at 121^0C 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 bacteria
Sterilized Samples were inoculated on NA media plates. These plates were incubated at 37^0C for 72hrs.The grown cultures were maintained by sub culturing periodically and preserved at refrigerator.

Antibacterial activity of isolates
Antibacterial activity of Endophytic isolates were screened

against *Shigella species* by agar well diffusion method.
In this method, test microorganism Shigella species was spreaded on sterilized NA plates and wells were prepared with the help of if sterilized cork borer. Isolated Endophytic bacterial culture were added in the prepared wells. Plates were incubated at 370 for 72 hrs. After 72 hrs zone of inhibition was observed and measured around the well.

Results

Fig 1: Collection of samples and isolation of Endophytic Bacteria

Isolation of endophytic microorganisms

From the Fresh body parts of grass total 16 endophytic bacteria were isolated from different regions of Pune shown in figure I and II and they were named as RB1, RB2.....RB16.

Antibacterial Activity of endophytic isolates

Isolated Endophytic bacteria RB1 to RB16 showed antimicrobial activity against Shigella species by agar well diffusion method. From RB16, RB10 showed highest antimicrobial activity against *Shigella* species and shown in Table I.

Table 1: Antibacterial activity of endophytic

Sr. No.	Isolates	Antibacterial activity (mm)
1	RB1	3
2	RB2	6
3	RB3	4
4	RB4	4
5	RB5	6
6	RB6	4
7	RB7	2
8	RB8	4
9	RB9	5
10	RB10	10
11	RB11	4
12	RB12	6
13	RB13	8
14	RB14	7
15	RB15	6
16	RB16	6

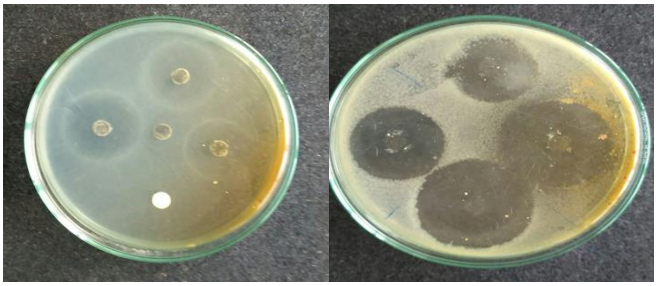

Fig I: agar well diffusion method isolates.

Discussion

Microbial endophytes from medical plants are reported as source of original secondary metabolites (Leiter *et al* 1950).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).

Conclusion

Plants contain endophytic microorganisms and they are able to produce secondary metabolites against *Shigella species* therefore they are useful in future clinical applications.

Reference

1. Aridason W, Lakshminarasimha LP, Status of plant driver sitt in India. 2017.
2. Bacon CW, Porter JK, Robbins JD, Luttrell ES. Epichloë typhina from toxic tall fescus grasses. Applied & Environmental Microbiology. 1977; 34:576-581.
3. Barengo N, Sieber TN, Holdenrieder O. Diversity of endophytic mycobiota in leaves and twigs of pubescent birch (*Betula pubescens*). Sydowia. 2000; 52:305-320.

4. Carroll GC, Carroll FE. Studies on the incidence of coniferous needle endophytes in the Pacific North West, USA. Canadian Journal of Botany. 1978; 56:3034-3043.
5. Clay K. Fungal endophytes of grasses. Annual Review of Ecology and Systematics. 1990; 21: 275-297.
6. Rodrigues KF, Hesse M, Werner C. Antimicrobial activities of secondary metabolites produced by endophytic fungi from Spondias mombin. Journal of Basic Microbiology. 2000; 40:261-267.
7. Rohrmann S, Molitoris HP. Screening for wood-degrading enzymes in marine fungi. Canadian Journal of Botany. 1992; 70:2116-2123.
8. Somogyi M. Notes on sugar determination. Journal of Biological Chemistry. 1952; 195:19-23.
9. Strobel GA, Yang X, Sears J, Kramer R, Sidhu RS, Hess WM. Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana. Microbiology. 1996; 142:435-440.