Evaluation of Endophytes for their Antimicrobial activity from Indigenous Medicinal Plants belonging to North Maharashtra region India .

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Abstract

Evaluation of some endophytes have been carried for their possible antimicrobial activity from various parts of medicinal plants belonging to Jalgaon Maharashtra (India).A total of seventy eight bacterial endophytes and one hundred fourty two fungal endophytes were isolated from the aerial and underground parts of selected medicinal plants .Fifteen positive endophytic bacterial isolates and fourteen positive endophytic fungal isolates possess antifungal and antibacterial activity respectively. Bacterial isolates KB4 from roots of roots of *P.glabra*, NB6 from stem *of E.globulus* and HB3 from rhizomes of C.*longa* have strong antifungal activity. Endophytic fungi AFR1, AFR4, AFR7 from roots of *A.vera* possess strong antibacterial activity against *S. typhi* in dual culture assay.

Keywords: Antibacterial activity**,** Antifungal activity, Endophytes, Medicinal plants.

Introduction

Endophytes are microbes that inhabits plant tissues in their life cycle without causing any apparent harm to their host [1].Their presence implied a symbiotic interaction, in all the plants investigated until now[2] .Endophytes were mentioned for first time by Bray in 19th century [3]. Endophytes have been found in nearly all plant families [4]. Dryfuss and Chapera [5] estimated that there may be at least one million species of endophytic fungi alone. Recently endophytes are viewed as outstanding source of secondary metabolites and bioactive antimicrobial natural products. Endophytic fungi are of biotechnological interest due to their potential use as genetic vectors , metabolites [6,7,8] and biological control agents[9,10,11,12].Present study was carried out by selecting indigenous medicinal plants growing in Jalgaon viz. *as Azadirachta indica* A Juss , *Curcuma longa* L., *Eucalyptus globulus* Dehnh ,*Musa paradiasica* L., *Pongamia glabra* Vent, *Aloe vera* L. *Morrayo konengi* L. Sprengel ., *Osimum sanctum* L.,Mant . Endophytic microorganisms from indigenous medicinal plants were evaluated for their antimicrobial activities.

Materials and Methods

Mature healthy plant materials were collected by sampling different parts of the selected medicinal plants, fifteen samples were taken from each tree five each from roots , inner bark and leaves, From each sample ten sub samples were separated further for isolation of endophytes. Samples were immediately brought to laboratory and were used within 24 hrs .Samples were washed in running tap water for 10 minutes to remove , soil particles and adhered debris , and finally washed with distilled water. Surface sterilization was done using methodology described by Petrini 1992[13] With some modifications .Samples were immersed in 70% ethanol 1-3 minutes and 4% aqueous solution of sodium hypochlorite 3 minutes again 1 minutes with 70% ethanol 1 minutes and 0.1% mercury chloride 2 minutes .Finally rinse with sterile distilled water three times. Samples tissues were selected by aseptic cutting using sterile knife and inner tissues were excised. Tissues were inoculated on to the water agar and Tryptic soy agar media for two to six days at 25^{o} C to isolate endophytic bacteria and for isolation of endophytic fungi inoculated on to the potato dextrose agar containing Streptomycin as to avoid bacterial growth and incubation allowed for 6-25 days at 25^{o} C.

Antifungal activity by Bacterial Endophytes

Antifungal activity was screened using dual culture method in which both endophyte and test fungi were inoculated in same media plate. Five days old culture disks (5mm diameter)of test fungi were inoculated at the centre of potato dextrose agar plate and endophytic bacteria were spot inoculated at three corner of the plate and incubated for four to eight days at 27^0 C. Antifungal activity was indicative as mycelia growth of test fungus prohibited in the direction of active endophyte, the level of inhibition was calculated by subtracting the distance (mm)of fungal growth in the direction of an antagonist colony from the endophyte growth radius. The width of inhibition zone between the pathogen and endophyte was evaluated as inhibition zone and ranked as .+,<2mm;++ >,2-10mm;+++ ,10 > mm[14].

Antibacterial activity fungal Endophytes

Antibacterial activity was screened using dual culture technique with some modification. Suspension of 24 hrs old culture of pathogenic bacteria was spread on sterile nutrient agar plate on to which five day old disc (5mm diameter) of endophytic fungi was kept and incubated at 27^0c for 24 -48 hrs. Antibacterial activity was calculated by measuring zone of inhibition produce by endophytic fungi against pathogenic bacteria.

Inhibition zone diameter index :+++> 10mm, ++5-10mm+<5mm –no zone [15]

Table 1: Antifungal activity by some positive endophytic bacteria from indigenous medicinal plants

Host	**Family**	**Tissue**	**Isolates**	**Positive**	**Antifungal activity**				
				Isolates	**An**	**Aa**	**Tk**	**Fo**	**Pf**
A. indica	Meliaceae	Ls	09	(01)NMB1	++	-	++	++	+++
		Rt	07	(01)NMB4	-	-	+++	-	-
		St	06	(01)NMB6	++	++	++	++	++
C.longa	Zingiberaceae	Rz	06	(01)HB3	+++	+++	+++	++++	++
E.globulus	Myrtaceae	Ls	07	(01)NB1	-	-	-	-	-
		St	05	(01)NB6	+++	+++	+++	+++	+++
		Rt	04	(00)	-	-	-	-	-
M.paradiasica	Musaceae	Ls	10	(01)BB1	+	+	+	++	+
		St	06	(02)BB2	++	-	-	+	+
		Rt	04	(02)BB5	-	-	++	-	-
P.glabra	Leguminoseae	Ls	08	(01)KB1	++	-	+	+	-
		St	06	(01)KB2	-	-	-	-	-
		Rt	06	(01)KB4	+++	+++	+++	+++	+++

a**)An:** *Aspergillus niger*, **Aa:** *Aspergillus avamori, Tk: Trycoderma konningi*, Fo**:** *Fusarium oxysporium,*

b) Pc: *Penicillium fumicalsuri*

c) Antifungal activity: +, < 2mm; ++, 2-10mm; +++, >10mm.

d) **Ls:** Leaves, St**:** Stem, **Rt:** Roots, **Rz:** Rhizomes.

Table2: Antibacterial activity by some positive endophytic fungi from indigenous medicinal plants.

Host	**Family**	**Tissue**	**Isolate**	**Positive**	**Antibacterial activity**					
				Isolates	**Ec**	**St**	**Bs**	**Sa**	**Pv**	**Pf**
A. vera	Liliaceae	Rts	20	(03)ALF1	-		+++	-	-	-
				ALF4			++	-	-	-
				ALF7			+++	-	-	-
C. longa	Zingiberaceae	Rz	08	(02) HF4	++		-	3.2	++	+++
				HF6	-		+	++	+	-
M. konengi	Rutaceae	Ls	20	(01) KTP1	-		-	++	-	+
		St	17	(01) KTP6	+		+	+	-	-
		Rt	15	(01) KTP8	+		+	+	+	-
M. paradiasica	Musaceae	Ls	07	(01) BBF5	+		+	-	-	+
		St	06	(00)	-		-	-	-	-
		Rt	05	(00)	-		-	-	-	-
O.sanctum	Labiatae	Ls	20	(03) TF3	+		-	-	+	+
				TF9	+		+	-	+	-
				TF5	++		++	+	+	+
		St	15	(01) TF1	++		+	+	-	+
		Rt	09	(01) TF17	+		+	+	+	+

a)Inhibition zone diameter index :+++> 10mm, ++5-10mm+<5mm –no zone

b) **Ec:** *Escherichia coli*, **St:** *Salmonella typhi*, **Bs:** *Bacillus subtilis*, **Sa:** *Staphylococcus aureus* ,

Pv: *Protease vulgaris*

c) **Ls:** Leaves, St**:** Stem, **Rt:** Roots, Rz**:** Rhizomes.

Results and Discussion

Plants have long provided mankind with a source of medicinal agents,with natural products once serving as source of all drugs[16].Recently biological controls or the uses of microorganisms or their secretions to prevent diseases offer an attractive alternative or supplement to disease management without the negative impact of chemical

control[17] .A total of seventy eight bacterial endophytes and one hundred and fourty two fungal endophytes were obtained from roots ,stem and leaf tissues of seven different medicinal plants used by local population of Jalgaon. KTP1from roots of *M. konengi,* TF5 from leaves of *O.sanctum* were strong candidates

Table 1 summarizes antifungal activity by bacterial endophytes .About fourteen positive Endophytic bacteria were screened for their antifungal activity against pathogenic fungi. Isolate KB4 from roots of *P.glabra,* NB6 from stem *of E.globulus* and HB3 from rhizomes of *C. longa* are potent as per antifungal activity was concerned this is followed by isolates NMB1,NMB6, BB1 from *A.indica* and *M.paradiasica,* rest other endophytic bacteria are less effective. Highest antifungal activity was recorded in endophytic bacteria KB4.

Table 2 shows antibacterial activity by fungal endophytes. fourteen positive endophytic fungi were obtained ,endophytic fungi HFR4 ,HFR6 from rhizomes of *C. longa* were powerful isolates for antibacterial activity against pathogenic microbes .Fungal endophytes AFR1,AFR4,AFR from roots of *A.vera* showed strong inhibition against *S.typhi* . Fungal endophyte KTP1 from roots of *M. konengi,* TF5 from leaves of *O.sanctum* found to have good antifungal activity. Other fungal endophytes were less active. Thus the activity spectra of endophytes were greatly different depending upon isolates, suggesting that several substances participated in antimicrobial activity.

Antifungal activities of plant endophytic fungi have been reported by some researchers[18,19,20,21,22].Gram negative endophytic bacteria with potent antibacterial activity from roots of *Solanum* sp was reported by Long , 2003[15]. Population density of endophytes seems to be highest in aerial tissues than the underground tissues. The diversity of endophytes obtained from healthy plant tissues suggests that an even broader flora of endophytes might be found across diverse plant species. These results suggest the presence of an active principle in the strains showing positive biological activities so needs to be enhancing production of the secondary metabolite of the interest. It is clear that endophytes isolated may be beneficial to their host .Antibacterial resistance especially among Gram negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search for alternative drug or natural antibacterial [22].This aspect of antimicrobial activity will be further investigated to enhance production of secondary metabolites of interest. Therefore, any information and research on endophyte plant symbiosis is of value. Recently biological controls or the uses of microorganisms or their secretions to prevent diseases offer an attractive alternative or supplement to disease management without the negative impact of chemical control [17].Plants have long provided mankind with a source of medicinal agents, with natural products once serving as source of all drugs [16]. Endophytic microorganisms are excellent sources of bioactive natural products that can be use to satisfy demand of Pharmaceutical and Medical Industries.

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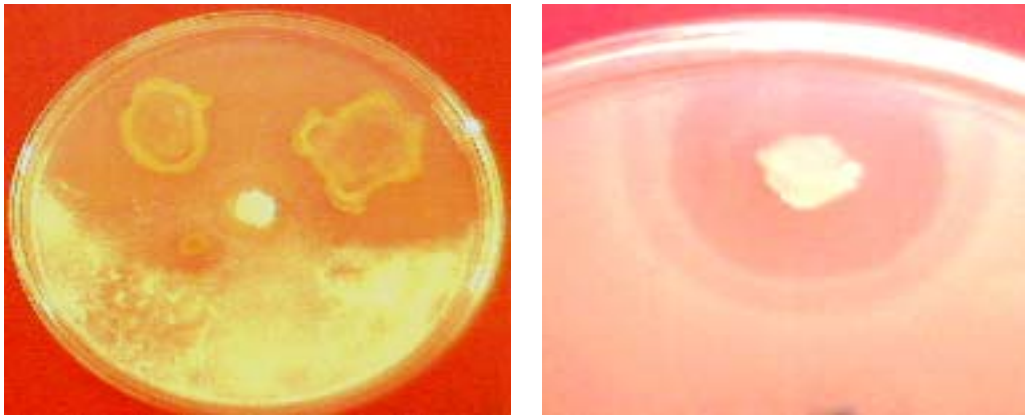
a. b.

Figure1.Endophytic fungi isolated from branch of *Morrayo konengi* (a) and roots of A.vera (b.)



a. **b.**

Figure1. a. Antifungal activity by endophytic bacteria KB4 against *Trycoderma konningi* and
b. antibacterial activity by endophytic fungi ALF1against *S.typhi*