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In vitro Screening of Selected Endophytic Fungi against Bacterial Leaf Blight of Rice Caused by *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

The present work is focused on isolation of endophytic fungi and its antibacterial activity against Bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). The secondary metabolites from endophytic fungi are useful in preventing the host from being attacked successfully by other microbes and pests. Biological control is an ecology-conscious, cost-effective, and sustainable alternative method in BLB management. The previously maintained *Xanthomonas oryzae* pv. *oryzae* (Xoo) cultures in the laboratory were used for the study. Selected endophytic fungi were screened against the Xoo to check for its antibacterial activity. Sixteen endophytic fungi were screened *in vitro* against Xoo. Among 16 endophytic fungi only fourteen endophytic fungi showed zone of inhibition ranging from 8-20mm in diameter against Xoo.

***Key words*:** Rice, *Xanthomonas oryzae* pv. *oryzae* (Xoo), Endophytic fungi, Bacterial leaf blight

Rice (*Oryzae sativa* L.) is one of the most important crop and a primary food source for half of the world's population, it belongs to the family Poaceae and it is the most widely cultivated food crop world over [1]. In world, annually more than 40% of the rice crop is lost owing to biotic stresses like insects, pests, pathogens and weeds. Among several diseases caused by bacteria, fungi and virus pathogens that devastate rice yields all over the world, some of the important diseases are Bacterial Leaf Blight (BLB) caused by (*Xanthomonas oryzae* pv. *oryzae*), Blast (*Magnaporthe grisea*), Sheath Blight (*Rhizoctonia solani*), Sheath Rot (*Sarocladium oryzae*) and Tungro virus [2].

Bacterial Blight of Rice is one of the most destructive disease caused by Xoo which limits the annual rice production in both tropical and temperate regions of the world [3]. In the tropics, damage is more severe than in the temperate regions [4]. It is one of the oldest known diseases and was first observed by the farmers of Japan in 1884. Subsequently, this incidence has been reported from different parts of the Asia, northern Australia, Africa and USA [5]. India has the largest area under rice cultivation (43 million hectares) and with production of 87.80 million tons, next to China. In India the bacterial blight disease was first reported in 1951 [6]. Among top 10 bacterial plant pathogens Xoo in fourth position after *Pseudomonas syringae*, *Ralstonia solanacearum* and *Agrobacterium tumefacient* [7].

The disease mainly occurs in the host plant at the seedling, vegetative and reproductive stages but bacterial leaf blight causes severe infection at the tillering stage resulting in yield loss up to 75% depending on weather, location and particular rice cultivar used [8]. Yield loss of the crop due to bacterial disease depends on the virulence of pathogen, host-pathogen relationship and different environmental factors. In India, BLB disease was observed in many important rice growing states like Andhra Pradesh, Bihar, Haryana, Kerala, Orissa, Punjab and Uttar Pradesh [9]. The bacterium exists in different races (or pathotypes) distinguished based on their behaviour on differential cultivars. New races are variable in bacterial virulence. Resistance to BLB is known to be widely different with rice cultivars [10].

Endophytic fungi are group of microbes which are synergistic to their host, colonizing asymptomatically in living, internal tissues of healthy plants and has attracted considerable attention for their ecological and biotechnological potential [11]. The secondary metabolites from endophytic fungi are useful in preventing the host from being attacked successfully by other microbes and pests [12]. The ability of endophytic fungi to produce various compounds such as antimicrobial substances, antitumor

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substances, enzymes and plant growth hormones have been demonstrated from several studies on endophytic fungi [13]. The endophytic fungi have been observed to inhabit or kill a wide variety of harmful disease causing microorganisms and are also a good source of antibiotics [14].

Various disease management practices have been employed to reduce damage caused by BLB such as chemical control, host-plant resistance, modification of cropping systems, and biological control. Chemical control are the ones that functions at low concentration by either killing or inhibiting the multiplication of the pathogen by blocking an important metabolic pathway and also readily translocate, stable in the plant system causing minimal damage to the environment. Chemical control of BLB in rice fields began in 1950s with the preventive application of Bordeaux mixture, other chemicals such as tecloftalam, phenazine oxide and nickel dimethyldithiocarbomate sprayed directly on plants [15]. Synthetic organic bactericides such as nickel dimethyl dithiocarbamate, dithianone, phenazine and phenazine noxide were also recommended [16]. Excessive use of chemicals also has a detrimental effect on the environment, farmers and consumer health [17].

Biological control is an ecology-conscious, cost-effective, and sustainable alternative method in BLB management [18]. Control of plant diseases by the use of antagonistic microorganisms can be an effective means. Interaction between biocontrol agent and plant pathogens has been studied extensively and application of biocontrol agents to protect some commercially important crops is promising [19]. However, biological control appears to be a suitable environment-friendly strategy for disease control and management [20]. The present study is focused on the isolation of endophytic fungi and its antibacterial activity against the plant pathogen Xoo.

MATERIALS AND METHODS

Isolation and identification of endophytic fungi

The endophytic fungi were isolated directly from leaves of the plant material (name of the plant) collected from Western Ghats of Karnataka and the samples were brought to the laboratory and washed with running tap water. The sample was surface sterilized with sodium hypochlorite solution for 30-60 seconds followed by sterile distilled water (SDW) for a minute and blot dry on sterile blotting paper, transferred on to potato dextrose agar (PDA) medium and incubated at 24±2°C for 5-7 days and observed for the colonies on the plates and the pure colonies were transferred on PDA slant [14]. The identification of endophytic fungi was carried out based on the cultural and microscopic properties using standard manuals.

In vitro screening of endophytic fungi by agar plug method against Xanthomonas oryzae pv. oryzae

The *Xanthomonas oryzae* pv. *oryzae* cultures used for the study were maintained in our laboratory which were identified by biochemical tests and molecular identification as mentioned in our publication [21]. The mycelial disc of endophytic fungi isolated from the plants of *Millingtonia hortensis* (Tree Jasmine), *Mimusops elengi* (Spanish Cherry), *Syzygium jambos* (Rose Apple), *Plectranthus amboinicus* (Doddapatre) and *Ocimum tenuiflorum* (Tulsi) collected from Western Ghats were screened for its antibacterial activity against Xoo isolated from infected leaf parts of diseased rice collected from different parts of Karnataka.

The selected endophytic fungi strains were grown on PDA Petri plate for 5-7 days at 24±2°C with alternate light and dark period. Later from seven-day old culture the fungal discs were pierced using a sterile cork borer of 5mm in diameter. The fungal discs were transferred to Muller-Hinton agar (MHA) plate which was priorly inoculated with bacterial strain. The cultures were kept for 12 h at 2-8°C for the diffusion of antimicrobial substance and thereafter the petri plates were incubated at 28°C for 24 h and the zone of inhibition was measured in millimetre (mm) and recorded [22].

Statistical analysis

All data of experiments were analyzed statistically, using SPSS software (version 20.0) and Microsoft Excel. The obtained data were further subjected to analysis of variance (ANOVA), and the means were analyzed using Duncan's new multiple range post test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Isolation of endophytic fungi

Isolation of endophytic fungi from medicinal as well as other plant results in the production of bioactive compound which has greater activity against various pathogenic microbes. Therefore, large production of these bioactive compounds in large scale must be necessary to fulfill the needs of agriculture and pharmaceutical industries [23]. The endophytic fungal strains were isolated from leaf parts of the plants *Millingtonia hortensis* (Tree Jasmine), *Mimusops elengi* (Spanish Cherry), *Syzygium jambos* (Rose Apple), *Plectranthus amboinicus* (Doddapatre) and *Ocimum tenuiflorum* (Tulsi) collected from Western Ghats. A total of 16 fungi were isolated and all the fungi belonged to the different division of fungi. The identification of fungi was done based on the cultural and microscopic properties using standard manuals [24] (Table 1). Our study correlated with Atiphasaworn *et al.* [25] where fourteen endophytic fungi were isolated from healthy leaves of *O. basilicum* var. *Thyrsiflora.* Similar work Rani *et al*. [26] was carried out by where twenty endophytic fungi were isolated from *Calotropis procera*.

Table 1 Isolation of endophytic fungi from plants collected from Western Ghats

Name of the plants	*Millingtonia hortensis* (Tree Jasmine)	*Mimusops elengi* (Spanish Cherry)	*Syzygium jambos* (Rose Apple)	*Plectranthus amboinicus* (Doddapatre)	*Ocimum tenuiflorum* (Tulsi)
Endophytic fungi	*Myrothecium* sp. *Aspergillus niger* *Aspergillus* sp. *Trichoderma* sp.	*Aspergillus terreus* *Trichoderma* sp. *Cladosporium* sp.	*Alternaria* sp. *Aspergillus niger* *Cladosporium* sp. *Trichoderma* sp.	*Trichoderma* sp. *Alternaria* sp.	*Trichoderma* sp. *Aspergillus niger* *Alternaria* sp. Sterile mycelia Sterile mycelia

Antibacterial activity of endophytic fungi against Xanthomonas oryzae pv. oryzae

Endophytes are rich in bioactive metabolites, which have major medical, agricultural, and industrial applications [27]. Endophytes have gained a lot of interest from scientists because of their ability to produce novel bioactive compounds [28]. Among sixteen endophytic fungi used except *Myrothecium and Cladosporium* all other fourteen endophytic fungi showed inhibitory zone ranging from 8-20mm in diameter against Xoo. The effective fungi were *Alternaria* sp. from *Plectranthus amboinicus*, *A. niger* from *Millingtonia hortensis*, *Trichoderma* sp. from *Millingtonia hortensis*, *A. niger* from *Syzygium jambos*, and *Alternaria* sp. from *Ocimum tenuiflorum* which inhibited moderately virulent strains of Xoo showing maximum inhibitory zone ranging from 10-20mm in diameter. The results demonstrated that some of the endophyte cultures were potent for inhibiting the growth of tested bacteria. However, it needs more investigation to disclose the possibility that those endophytes would be used for biocontrol of the related plant diseases by introducing them into the crops (Table 2) (Fig 1).

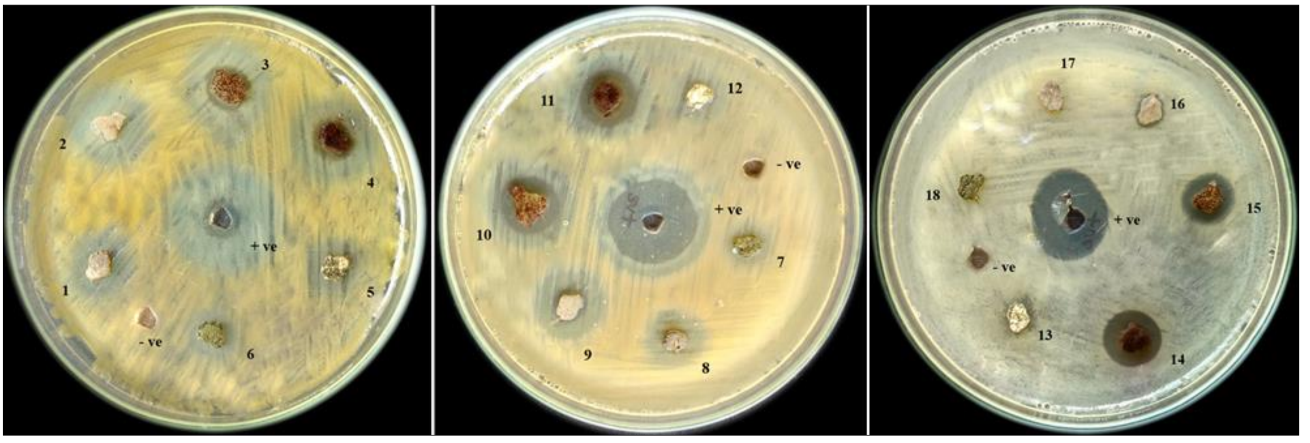

Fig 1 Antibacterial activity of Endophytic fungi against *Xanthomonas oryzae* pv. *oryzae*

Table 2 Antibacterial activity of endophytic fungi against *Xanthomonas oryzae* pv. *oryzae*

Endophytic fungi	Antibacterial activity of endophytic fungi against Xoo (mm)		
	XooMB53	XooMB69	XooMB70
Myrothecium sp.	0^{a}	0^{a}	0^{a}
Aspergillus niger	$15.33^{klm} \pm 0.57$	$15.33^{klm} \pm 0.57$	$15.33^{klm} \pm 1.52$
Aspergillus sp.	$10.33^{ef} \pm 0.57$	$10.33^{ef} \pm 0.57$	$13.66^{ij} \pm 1.15$
Trichoderma sp.	$14.66^{jk} \pm 0.57$	$16.33^{m} \pm 1.52$	$17.66^{n} \pm 1.15$
Aspergillus terreus	$13^{hi} \pm 0$	$15^{kl} \pm 0$	$15.66^{klm} \pm 1.15$
Trichoderma sp.	0^{a}	0^{a}	0^{a}
Cladosporium sp.	0^{a}	0^{a}	0^{a}
Alternaria sp.	$12.33^{h} \pm 0.57$	$18.66^{n} \pm 0.57$	$20^{o} \pm 1$
Aspergillus niger	$15^{kl} \pm 0$	$16^{lm} \pm 1$	$16.33^{m} \pm 0.57$
Cladosporium sp.	0^{a}	0^{a}	$11^{fg} \pm 1$
Trichoderma sp.	0^{a}	$6.33^{b} \pm 0.57$	$9^{cd} \pm 1$
Trichoderma sp.	0^{a}	$8^{c} \pm 1$	$10.33^{ef} \pm 0.57$
Alternaria sp.	$16^{lm} \pm 1$	$13^{hi} \pm 1$	$16.33^{m} \pm 0.57$
Trichoderma sp.	0^{a}	$12^{gh} \pm 0$	$12.66^{hi} \pm 1.15$
Aspergillus niger	0^{a}	0^{a}	0^{a}
Alternaria sp.	$15.33^{klm} \pm 0$	$14.66^{jk} \pm 0.57$	$18^{n} \pm 0$
Sterile mycelia	0^{a}	0^{a}	0^{a}
Sterile mycelia	0^{a}	0^{a}	0^{a}
Positive	$22^{p} \pm 1$	$21.33^{p} \pm 0.57$	$23.66^{q} \pm 1.15$
Negative	0^{a}	0^{a}	0^{a}

Means of three replications followed by the letters significantly different according to Duncan's multiple range tests (DMRT). The superscripts in a column significantly different ($p \leq 0.05$)

CONCLUSION

The present study clearly demonstrates that bacterial leaf blight disease is prevailing in all the surveyed regions of the state with varied degree of disease incidence. This different degree of disease incidence may be due to the prevailing agro climatic conditions and nature of host cultivar. Biological control assumes special significance in being ecology conscious, cost-effective alternative strategy for bacterial blight management. There is increase in number of studies focusing on fungal endophytes because they benefit plant growth and protection by the production of bioactive secondary metabolites. Though biological control agents often perform inadequately under field conditions it can be still accepted as an alternative to chemical pesticides.

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