

Isolation and Identification of *Xanthomonas oryzae* pv. *oryzae* the causal agent of rice bacterial leaf blight and its activities against of six medicinal plants

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ABSTRACT

The antibacterial properties of the leaf extracts of *Morinda coreia* Buch.-Ham., *Datura innoxia* Mill., *Ocimum tenuiflorum* L., *Calotropis gigantea* (L.) Dryand., *Vitex negundo* L. and *Pongamia pinnata* (L.) Pierre and the fruit extract of *Morinda coreia* and *Datura innoxia* against the bacteria *Xanthomonas oryzae* pv. *oryzae* isolated from Paddy fields. The pathogen *Xanthomonas oryzae* pv. *oryzae* commonly causing the bacterial leaf blight disease in rice. The antibacterial assay was determined by well diffusion method. Four different solvents such as hexane, ethyl acetate, ethanol and aqueous were used. Among the four solvents ethyl acetate extracts of all the treated plant leaves and fruits showed maximum significant inhibitory activity followed by ethanol extract, hexane extract and aqueous extract.

Key words: Antibacterial activity, *Xanthomonas oryzae* pv. *oryzae*, bacterial leaf blight disease,

INTRODUCTION

Annually more than 40% of the world's rice crop is lost owing to biotic stresses like insects, pests, pathogens and weeds (Hossain, 1996). Among several diseases caused by bacterial, fungal and viral pathogens that devastate rice yields all over the world, bacterial leaf blight (BLB) (*Xanthomonas oryzae* pv. *oryzae*), blast (*Magnaporthe grisea*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*) and tungro virus are the most important (Velusamy *et al.*, 2006).

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is the most important and oldest known bacterial disease of rice in Asia (Hasan Naqvi *et al.*, 2014) and most serious bacterial diseases in many of the rice growing regions of the world (Xu *et al.*, 2010). The disease was first observed by the farmers of Japan in 1884 (Tagami and Mizukami, 1962). Crop losses of 10%-20% in moderate conditions or severe losses of up to 50% in highly conducive conditions have been recorded in several Asian and Southeast Asian countries (Mew, 1993). Globally, its incidence has been reported from different parts of Asia, northern Australia, Africa and the United States. In India, BLB disease has been observed in most important rice growing states like Andhra Pradesh, Bihar, Haryana, Kerala, Orissa, Punjab and Uttar Pradesh. The disease occurred in an epidemic form during 1998 in the Palakkad district of Kerala (Venkatesan and Gnanamanickam, 1999) and since then has been observed in severe proportions almost every year. BLB disease reduces yields and yield stability. Some management tactics, such as host plant resistance based on single genes, may not be durable in the field and might lead to frequent varietal breakdowns.

Medicinal plants represent a rich source of antimicrobial agents. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Kagale *et al.*, 2004). The effects of plant extracts on bacteria have been studied by a very large number of

researchers in different part of the world (Reddy *et al.*, 2001; Ateb and Erdo Urul, 2003). Much work has been done on ethno medicinal plants in India. Interest in a large number of traditional natural products has increased (Muneer *et al.*, 2007).

MATERIALS AND METHODS

Isolation of BLB pathogen from rice plant

Xanthomonas oryzae pv. *oryzae* (Xoo), the causal agent of bacterial leaf blight was isolated from the diseased plants of rice. Infected leaf pieces 28×7 mm of rice were excised with sterile scalpel. The leaf surface was sterilized with 1% Clorox (Sodium hypochlorite) for three minutes and then washed with sterile distilled water (SDW). Leaf pieces (6-7) of rice leaves after drying on sterile blotting paper were transferred to nutrient agar (NA) medium and incubated at room temperature (25-27°C) for 72hrs (Jabeen *et al.*, 2012). The emerging colonies were sub-cultured onto NA plates and to get pure culture. Cultures were preserved for longer duration (at 4°C) on NA slants.

Identification of bacterial pathogen from rice

Pure culture was identified at Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Medicinal Plants Collection and Sterilization

Morinda coreia, *Datura innoxia*, *Ocimum tenuiflorum*, *Calotropis gigantea*, *Vitex negundo* and *Pongamia pinnata* was collected from Tiruchirappalli region, Tamil Nadu. Fresh plant leaf materials and fruits were washed under running tap water followed by sterilized distilled water, air-dried and then powdered with the help of sterilized pestle and mortar. The powders were further subjected for different extraction protocols as given below:

Preparation of plant extracts

Aqueous extraction

10 g of air-dried powders of respective plants were boiled in 100 ml distilled water till one fourth of the extract initially taken was left behind after evaporation. The solution was then filtered using muslin cloth. Filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was again filtered using Whatman Filter No. 1 under strict aseptic conditions and the filtrate was collected in fresh sterilized bottles and stored at 4°C until further use.

Organic solvent extraction

10 g of air-dried powders were thoroughly mixed with 100 ml organic solvent (viz., Hexane, Ethyl acetate and Ethanol). The mixture was placed at room temperature for 24 h on shaker with 150 rpm. Solution was filtered through muslin cloth and then re-filtered by passing through Whatman Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of crude extracts for each type of organic solvent were prepared by mixing well the appropriate amount of dried extracts with dimethyl sulphoxide (DMSO) to obtain a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized bottles until further use.

Antibacterial screening on agar well diffusion method

Six medicinal plants extracts (hexane, ethyl acetate, methanol & aqueous) were screened for antibacterial activity by agar well diffusion method with sterile cork borer of size 6mm. The cultures of 24hrs old grown nutrient agar (NA) were used for inoculation of bacterial strain on NA plates. NA medium was poured into a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 50µl of hexane, ethyl acetate, ethanol and aqueous extracts of six different plant extracts were introduced into the each well, after inoculation the setup were incubated in room temperature at 24hrs. The antibacterial activity was evaluated by measuring zones of inhibition of bacterial growth surrounding the plant extracts. The complete antibacterial analysis was carried out under aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

RESULTS AND DISCUSSION

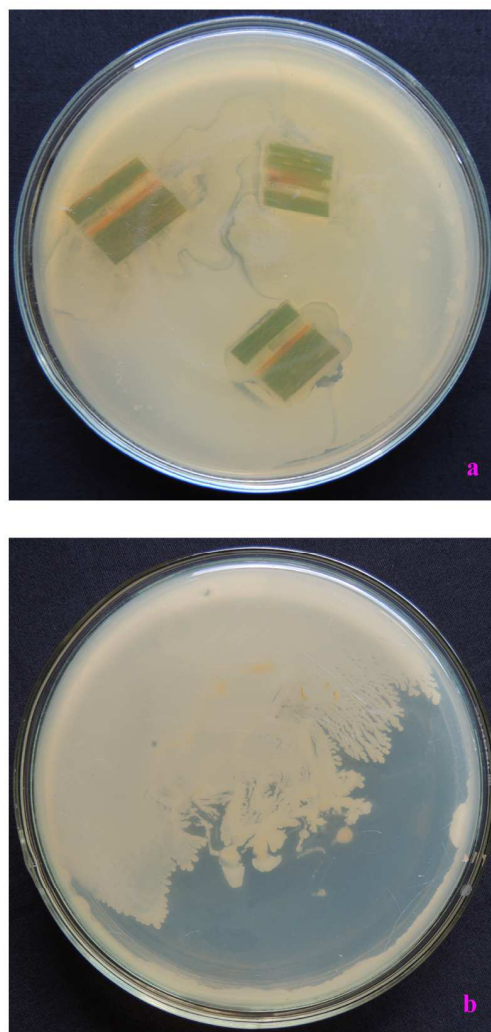
Isolation of bacterial pathogen from rice

The infected leaf samples of rice having Bacterial Leaf Blight symptoms were collected. Infected leaf samples when plated gave light yellow, mucoid, round and smooth bacterial colonies (Fig. 1) on nutrient agar medium (NA) after 48-72 hours incubation at 28±2°C. The emerging colonies were sub-cultured onto NA plates and to get pure culture and maintained on NA slants. The pure culture was identified and authenticated at Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Antibacterial screening on agar well diffusion method

Antibacterial activity of six medicinal plants extracts (Table 1) was assayed and the effect of plant extracts on the growth of *Xanthomonas oryzae* pv. *oryzae* was observed. The data revealed that significant reduction in growth of *Xoo* was observed with extracts of six medicinal plants and the extracts showed significant differences in their efficacy. Among all the six plant ethyl acetate extracts, 100% plants showed inhibition growth of *Xoo* over control. *Morinda coreia* fruit ethyl acetate extract (28.33 ± 1.88) showed exceptionally prominent activity followed by ethyl acetate extract of *Vitex negundo* leaf (17.33 ± 1.52) and ethanol extract of *Datura innoxia* (16.66 ± 0.57) leaf and fruit extracts also good activity.

Figure 1: Isolation of *Xanthomonas oryzae* Pv. *oryzae* Bacterium



a. Isolation of *Xanthomonas oryzae* bacterium on NA medium

b. Isolation of *Xanthomonas oryzae* colonies on NA medium

Many plant species have been reported to be antibacterial and this property can be utilized for the management of bacterial diseases (Narasimhan *et al.*, 1995). In the present study, we have shown that the fruit extracts of *M. coreia* followed by leaf and fruit extracts of *D. innoxia*, and leaf extract of *V. negundo* have profound inhibitory effect on the growth of *Xoo*. For instance, leaf extracts of *Artabotrys hexapetalus* highly inhibited the growth of *Xoo* (Velusamy *et al.*, 2013). There is considerable evidence that solvents improve the solubility of biologically active compounds compared to water (Rauha *et al.*, 2000).

Table 1: Inhibition activity of the six medicinal plants against *Xanthomonas oryzae* pv. *oryzae* (Xoo)

Type of Extract	Name of the Plants Zone of Inhibition (in mm diameter)							
	<i>Morinda coreia</i>		<i>Datura innoxia</i>		<i>Calotropis gigantea</i> (Leaves)	<i>Vitex negundo</i> (Leaves)	<i>Ocimum tenuiflorum</i> (Leaves)	<i>Pongamia pinnata</i> (Leaves)
	Fruits	Leaves	Fruits	Leaves				
Hexane	9±0.81	NI	10±0	8.33±0.57	NI	NI	NI	9.33±1.52
Ethyl acetate	28.33±1.88	9.33±0.57	12.66±0.57	15.33±0.57	11.33±1.52	17.33±1.52	9.33±1.52	12.33±0.57
Ethanol	9±0.81	NI	16.66±0.57	16.66±0.57	11.33±0.57	11.66±1.52	9.66±0.57	11.33±0.57
Aqueous	NI	NI	NI	14.66±1.52	8.66±1.15	NI	NI	NI
Control	NI	NI	NI	NI	NI	NI	NI	NI

*Mean of three values ± Standard Deviation, "NI" – No Inhibition was observed

CONCLUSION

The current scenario of antibiotics is very threatening with significant emergence of resistance among bacterial pathogens against available antibiotics. In our study, ethyl acetate proved to be a good solvent in extracting the inhibitory substances from the tested plant species as it showed maximum inhibitory activity against the *Xoo* organism.

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