INDUCTION OF RESISTANCE TO *PYRICULARIYA ORYZAE* IN UNDERLOWLAND RICE ECOSYSTEM BY SILICATE SOLUBILIZING BACTERIA

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ABSTRACT

Pyricularia oryzae is a phytopathogenic fungi plays major role in rice blast diseases worldwide. Silicon plays an essential role in providing tolerability to various abiotic stresses and augmenting plant resistance against diseases. However, there is a paucity of reports about the effect of silicon on plant pathogens. In general, the effect of silicon on plant resistance against phytopathogenic diseases is measured to be due to either physical defense or increased biochemical defense. The current study explains the agricultural importance of silicon in plants, refers to the control of plant pathogens by silicon solubilizing bacteria and the plant defence mechanism. Out of these, 78 bacterial isolates were identified from soil collected from various areas of Mayiladuthurai district. Amongst these 6 bacterial isolates were commonly found in tested areas which are analysed by silicate solubility efficiency. The highest silicate (zone diameter 44 mm) solubilization (zone diameter 55 mm) was observed for *Bacillus mucilaginosus* .Dual culture antagonistic assays were carried out by using these bacterial isolates against plant pathogenic fungi *Pyricularia oryzae'.* The pH, Electrical conductivity and Silicate quantity in silicon solubilizing treated soil was analysed. By comparing other isolates, *Bacillus mucilaginosus* shows high silicon content 238.16 ± 6.65 respectively. The Mean zone of inhibition of these bacterial isolates against the pathogenic fungi ranged between 8 mm to 22 mm. The largest zone of inhibition against all six bacterial strains was recorded for bacterial isolate *Bacillus mucilaginosus* followed by *Burkholderia Eburnean*. The highest mean zone inhibition of plant pathogen *Pyricularia oryzae by silicate solubilizing bacteria was found to be Bacillus mucilaginosus* (23 mm).These bacterial isolates will be further investigated for their plant growth promoting activities in the future.

Key words: Rice blast, Plant pathogen, silicate solubilizing bacteria, *Bacillus mucilaginosus etc*

INTRODUCTION

In India, Tamil Nadu is the leading state in rice production, as this State is endowed with all favourable climatic conditions suitable for rice growing. Rice is the most stable cereal crop plant in the world, feeding approximately half of the global population grown. The productivity of rice can be greatly enhanced by providing additional nutrient inputs and through effective control of disease incidence. (Mukamuhirwa *et al*., 2019)

Rice blast disease caused by *Pyricularia oryzae*, a plant pathogen is one of the most important fungal diseases of rice crop, causing loss of upto 90% in almost all the rice growing countries. It was first recorded in China (1637) as rice fever disease and was later in Japan (1704) (Wang, 2009). This phytopathogen is presently reported to be found in over 85 countries worldwide. The fungus particularly attacks the aerial parts of the rice plant at any stage of growth and any parts of plants. The infection occurs on the node below the panicle called neck blast which cause breakage of the panicle or poorly developed grain (Webster *et al*., 1992) and cause significant yield loss (Suzuki, 1975; Baker *et al*., 1997).

Plants absorb various mineral elements as nutrients for their ideal growth (Zhao *et al*., 2014). Among the various minerals, Si is considered a non-essential element; a recent study has proved its beneficial role in quality production of rice (Ma *et al*., 2007).Based on the literature, two mechanisms in which silicon can reduce the severity of Plant diseases have been reviewed. The first one is associated with an accumulation of absorbed silicon in the epidermal tissue acting as a physical barrier (Gutierrez-Barranquero *et al*. 2012), and the second one is related to an expression of metabolic or pathogenesis-mediated host defense responses (Song *et al*. 2016).

Eventhough, Silicon (Si) is the second most abundant element on earth. It remains unavailable for plants' uptake due to its poor solubility. Silicate solubilizing bacteria (SSB) can play an efficient role not only in solubilizing insoluble forms of silicates and also involved in induction of systemic resistence (ISR) against plant pathogens.

The current study focused on isolation and characterization of silicate solubilizing bacteria from paddy soil and evaluation of antagonistic activity against plant pathogen '*Pyricularia oryzae'*

MATERIALS AND METHODS

Collection of soil sample

Soil samples were collected from fields under routine cultivation of rice (high silicate accumulaors) from kuthalam, sirkali, Therizhandur, Manalmedu, Moovalur in mayiladuthurai district. One Kg of each soil sample was collected at a depth of 15 cm from top soil. The samples were then air dried and ten gram of each air-dried soil sample was transferred to 100 mL of sterile distilled water in a 250 ml Erlenmeyer flask separately. The samples were then incubated on a rotary shaker (120 rpm) for 30 min at room temperature as described by Naureen *et al*.,2015. Serial dilutions of each soil sample, ranging from 10^{-1} to 10^{-5} was prepared in sterile water. One mL aliquot of the appropriate dilution (10^{-5}) was spread on plates containing Luria Bertani (LB) agar. Plates were incubated at 30˚C ± 1˚C for 24 - 48 h and colonies were examined. Morphologically distinct colonies were counted, selected, purified and maintained in LB broth amended with glycerol (Naureen *et al*.,2009)

Morphological Characterization

Selected bacterial isolates were purified and plated on LB agar plates. Morphological characteristics were studied for pure single colonies ((Naureen *et al*.,2009))

Silicate Solubilization

Bacterial isolates from paddy soil samples were then spread on agar plates containing silicate medium with 0.25% insoluble magnesium trisilicate (Bunt and Rovira, 1955). Plates were incubated at a temperature of 30˚C ± 1˚C for 36 - 72 h. After the incubation period, the bacterial colonies exhibiting clear zones around them were selected for further investigations. Percentage of silicate solubilizing bacteria was checked and calculated as follows.

% of silicate solubilizers = Number of silicate solubilizing bacteria/total bacteria × 100

Analysis of Si Content of the Plant

The method described by Kang *et al*.,2017 was followed to quantify the Si content of the plant. In brief, 0.5 g of the lyophilized crushed powder was soaked in 0.5M HCl and rinsed through double distilled water before oven drying. A mixture of nitric acid, sulfuric acid, and perchloric acid (10:1:4 v/v/v) was subjected through the sample. The digested sample obtained was then analyzed.

Determination of Soil pH and Electrical Conductivity (EC)

The pH of the soil was determined both before and after the pot experiment. Before the experiment, 200 g soil per pot (10 X 10 cm) was filled in six pots. A randomly selected amount (0.2–2 g) of either magnesium trisilicate ($Mg_2O_8Si_3$) or calcium phosphate $Ca_3(PO_4)_2$ was mixed in each pot, and the pH of each pot was noted. After the experiment with silicate solubilizing bacteria, samples were collected, pH and EC were recorded. The method described by Jackson,1958 was used for the determination of EC of soil, while the procedure described by Kalra, 1995 was used for the determination of pH.

Antagonistic Assays

Antagonistic activity of selected bacterial isolates was checked against pathogenic fungi *Pyricularia oryzae* using dual culture assays as described by Naureen *et al*. 2009 and Hassan *et al.* 2010. Briefly, a 10 mm disc of an actively growing pure culture of pathogenic fungi grown on potato dextrose agar (PDA) was placed at the centre of a Petri dish containing appropriate test medium (rice flour agar RFA). A circular inoculum of selected bacterial isolates was made with a 6 cm diameter Petri dish dipped in a suspension of bacterial broth culture (5 × 109 -cfu·mL−1) and placed around the fungal disc. Plates were incubated for one week at 30˚C ± 1˚C and mean zone of inhibition was recorded after 7 days.

Statistical Analysis

Each experiment was carried out thrice and means were calculated

RESULTS AND DISCUSSION

Table 1: Morphological characterization of bacterial isolates commonly found from various areas in Mayiladuthurai

Serial no	Microorganism	Colony Morphology	Gram reaction	Cell Morphology
1	*Burkholderia eburnea*	White, shiny with smooth edges	-ve	Rod shape
2	*Bacillus mucilaginosus*	Fuzzy white, rough, opaque	-ve	Rod shape
3	*Enterobacter ludwigii*	Light gray smooth colonies	-ve	Rod shape
4	*Bacillus*	White, smooth	+ve	Rod shape

	amyloliquefaciens	circular colonies		
5	*Enterobacter asburiae*	Smooth irregular round to rough " Cauliflower" type colonies	-ve	Rod shape
6	*Pseudomonas fluorescens*	White, smooth edges, convex	-ve	Rod shape

Soil samples were collected during April to June 2019 from various areas in mayiladuthurai district from fields that had been long under cultivation of rice which are rich in minerals. A total of 78 bacterial isolates were isolated from these soil samples and maintained as pure cultures. Each bacterial strain was checked for silicate on respective medium. Out of bacterial isolates 6 bacterial isolates are commonly found in soil collected from various areas and It was analysed for silicate solubilizing efficacy. The highest silicate (zone diameter 44mm) were observed for Bacillus mucilaginosus while the lowest silicate solubilization was observed for Pseudomonas fluorescens (zone diameter 9 mm) shown in Table 1

Table 2: Silicate solubilizing efficacy of bacterial isolates

S.no	**Bacterial Isolate**	**Silicate Solubilization Zone (mm)**
1	*Burkholderia Eburnean*	34
2	*Bacillus mucilaginosus*	44
3	*Enterobacter ludwigii*	21
4	*Bacillus amyloliquefaciens*	18
5	*Enterobacter asburiae*	12
6	*Pseudomonas fluorescens*	9

Efficient silicate solubilizing bacteria can help release other essential nutrients in soil This can be directly due to solubilization of other minerals by silicate solubilizing bacteria or indirectly due to solubilized silicon. It has been previous reported that the solubilized Si improves availability of phosphorus to plants by competing with P fixation sites in soil (Muralikanna , 1998).. Thus Si acts as a substitute for P in plant system. Silicon is reported to increase the availability of Phosphorus indirectly by decreasing the availability of Fe and Mn in plants (Sahebi *et al*.,2015)

Table 3: Determination of pH, electrical conductivity, Silicon content in rice plant after treatment by silicon solubilizing bacterial isolates

Treatment by Bacterial isolates	pH	EC (ds m^{-1})	Si(mg Kg^{-1} D.W)
Control	5.07 ± 0.01	0.63 ± 0.01	152.23 ± 2.62
Burkholderia Eburnean	5.42 ± 0.17	0.98 ± 0.01	188.59 ± 5.03
Bacillus mucilaginosus	6.46 ± 0.04	1.68 ± 0.03	238.16 ± 6.65
Enterobacter ludwigii	5.42 ± 0.33	1.35 ± 0.03	175.87 ± 3.20
Bacillus amyloliquefaciens	5.11 ± 0.03	1.25 ± 0.05	127.33 ± 4.05
Enterobacter asburiae	4.96 ±0.01	0.80 ± 0.07	139.56 ± 5.51
Pseudomonas fluorescens	4.65 ± 0.03	0.75 ± 0.04	124.69 ± 3.97

EC: Electrical Conductivity, ds: deciSiemens, D.W: Dry Weight.
Assay was performed in triplicates. Values are expressed as mean ± SD

The present study showed that silicate solubilizing bacteria significantly increased Si content in rice plant on insoluble silicate ($Mg_2O_8Si_3$) based soil compared with the bacterial isolate treatment. The Preliminary tests of pH revealed that increase in silicate (Mg2O8Si3) concentration significantly increase the trend of pH. Addition of 0.4 g of ($Mg_2O_8Si_3$) to 200 g experimental soil per pot after 24 h showed pH values ranging from 5.0–6.0. The pH value of the experimental soil remained constant with amendment of Ca_3 $(PO_4)_2$. After the experiment with silicate solubilizing bacteria, the pH value of various bacterial treated soil was significantly higher compared with the control, while electrical conductivity was significantly higher in the *Bacillus mucilaginosus* treated soil compared with the control (Table 3).

Moreover, various factors such as pH, Electrical conductivity, temperature and time affect mineral dissolution (Xiao *et al*., 2015). Various factors such as pH, dissolved silicon concentration, and the activity of bacteria also affect the dissolution rate of SiO_2 (Javaheri *et al*., 2015). Since the present investigation proved that the silicon solubilizing bacterial activity thrives better at the pH ranging from 5–6.4, the microbes might have actively participated in the Si dissolution. Further studies are needed to test the potential of SSB for Si dissolution in field condition, for promoting plant growth and yield.

Table 4: Inhibition of phytopathogenic fungi '*Pyricularia oryzae'by Si solubilizing bacterial isolates*

Bacterial Isolates	Mean Zone of inhibition after 7 days(mm)
Burkholderia Eburnean	18
Bacillus mucilaginosus	23
Enterobacter ludwigii	16
Bacillus amyloliquefaciens	13
Enterobacter asburiae	12
Pseudomonas fluorescens	8

In addition to increasing the availability of Si, P and K in soil, the silicate solubilizing bacteria provide a very efficient system of biological control of plant pathogenic fungi. These bacteria can not only directly combat phyto-pathogenic fungi but indirectly by release of Si in soil which in turn induces disease resistance in plants either by acting as a physical barrier or by acting as a modulator of host resistance to pathogen. Si is deposited beneath the cuticle to form a cuticle-Si double layer which mechanically impedes penetration of fungi and thus disrupts the infection process

Almost all of the bacterial isolates showed at least some antagonistic activities against the fungal pathogen '*Pyricularia oryzae'*. Highest antagonistic activity was observed in case of bacterial isolate *Bacillus mucilaginosus* as shown by maximum zone of inhibition observed against tested pathogen followed by bacterial isolate *Burkholderia Eburnean*, *Enterobacter ludwigii*, *Bacillus amyloliquefaciens*, *Enterobacter asburiae and Pseudomonas fluorescens* respectively. There are several ways by which these silicate solubilizing bacteria can antagonize fungal pathogens. These include production of hydrolytic enzymes, siderophores, HCN and antibiotics (Hassan *et al*., 2010). The antagonistic potential of these bacteria is an important ability of practical utility in development of biopesticides against the fungal pathogens.

CONCLUSION

Silicates solubilizing bacteria plays a very eminent role in solubilizing silicon and make its availability to plant growth. It also enhance the plant defence mechanism against *Pyricularia oryzae* , a major rice plant pathogen. Silicate solubilizing bacterial strains isolated from various areas will be further investigated against various plant pathogens and hope for a possible development of biofomulations for commercial purpose.

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