Evaluation of antagonistic activity of *Chromobacterium violaceum* isolated from the paddy fields of Edathua, Kerala

Abstract: The soil surrounding the plant root, which has direct impact on plant by different microorganisms is known as the rhizosphere. This study focussed on the isolation, identification, and screening of different rhizosphere bacteria in the rice-growing regions of Edathua, Kerala. These rhizobacteria were first isolated on Kings B Agar and Nutrient Agar media. Gram staining is used to analyze the pigmentation property, and its antagonistic properties were screened by dual culture method. The bacteria were determined to be *Chromobacterium violaceum* after identification by partial gene sequencing using the 16S rDNA method. *Chromobacterium violaceum* is not typically considered as a common inhabitant of the rhizosphere region of paddy field, but in this study it has been found in rhizosphere soil of paddy. It was further established that the chosen pigment, isolate possesses the antagonistic capacity to generate non-volatile chemicals that inhibit the test pathogen (Fusarium sp.).This antimicrobal and antifungal properties of bacteria helps them to compete with other pathogenic microorganism in its environment and further shows its potential to be exploited as a natural ecofriendly biocontrol agent.

Index Terms **- Rhizosphere,Antagonism,Chromobacterium,Plant Growth Promoting Bacteria**

I. INTRODUCTION

Agriculture sector plays a pivotal role in growth and development of the country, by providing a livelihood to the people, sustainable agriculture also has contribution to world market, export and thereby keeping healthy communication between nations, provides labour to the people and thereby ensures the growth and development of the country. In India 72 percentage of total working population is a part of agricultural sector which makes it a dominant sector in Indian economy (Amarnath et al., 2009). Asia's food security mainly depends on irrigated rice fields, which accounts more than seventy five percent of the total rice production. Rice occupied a prime position in Kerala's agriculture. Population pressure also demands for the increase in the rate of the rice production (Durga et al., 2013). Edathua is a village in Kuttanad, Alappuzha district of Kerala which is well known for agriculture particularly paddy cultivation and is the main occupation of majority of people. Of the 2229 hectres of the total area 2000 hectres of land is used for agricultural purpose. 1620 hectres of land alone is used for paddy cultivation. Paddy, coconut, plantain, vegetables, etc. are the major crops cultivated in this land area. The unique environment in entire region provides cultivation of variety of crops (Santhi et al., 2019).

Rhizosphere is regarded as the 'hotspot' for the intense microbial activity and its colonization. Rhizobacteria are a consortium of total rhizosphere bacteria which is having both positive and negative influence on plant. The PGPR promotes plant growth by providing a disease free microenvironment with its antagonistic activity, siderophore and different enzymes which solubilises insoluble form of potash and phosphorus. Also help the plants by protection from diseases by producing anti-biotic and pathogen depressing substances (Kamnev et al., 2000). The rhizosphere microbes produce several chemical substances against the plant pathogens which completely inhibit the overall activity of the corresponding plant pathogen. PGPR strains act as an inhibitory factor for various pathogenic microorganisms through the production of some metabolites which stops the further growth of the particular pathogen. The microorganisms inhibit the phytopathogens by competing for the nutrient and space, producing bacteriocins, lytic enzymes, and antibiotics. (Indranil Singh, 2018). The present study is designed to isolate and identify the rhizosphere bacteria which have the potential to outcompete with the soil borne plant pathogens in their natural rhizosphere soil environment.

II. NEED OF THE STUDY

Millions of people around the world rely on paddy (Oryza sativa) as a staple crop to ensure their food security.However, paddy production is increasingly threatened by factors like emergence of soil-borne pathogens that cause significant yield losses. These pathogens frequently endure in the rhizosphere, taking advantage of soil properties that facilitate their development and dissemination.In recent years, biological control strategies have gained attention as eco-friendly and sustainable alternatives to chemical pesticides. Soil-borne microorganisms, particularly those inhabiting the rhizosphere, play a crucial role in suppressing pathogenic agents through various antagonistic mechanisms. Despite the promising potential of rhizosphere microorganisms, there remains a gap in identifying and evaluating indigenous microbial strains with strong antagonistic properties specific to paddy cultivation. Investigating these beneficial microbes could provide

innovative solutions for integrated disease management, promoting healthier crops and improved yields while minimizing chemical inputs.

III. RESEARCH METHODOLOGY

3.1. Isolation and identification of rhizobacteria

The soil samples were collected from fields cultivated with paddy (*Oryza sativa* L.) from Edathua village in Kuttanad, Alappuzha. Two samples are collected in sterile plastic bags for the analysis purpose. Of the two samples, one sample is collected along with intact root system of paddy from the field which is the rhizosphere soil and the second sample is collected from the area near to the rhizosphere. About 250g of soil along with intact root system is collected in plastic bags and tied them tightly and each sample systematically labeled with date, place, sample number, and sample type.

Preparation of medium

Preparation of Nutrient Agar

For solid media preparation

Peptone powder	:	5g
Beef extract	:	3g
NaCl	:	5g
Agar	:	15g
Distilled water	:	1000ml

Preparation of King's B Agar Medium

For solid media preparation

Peptone powder	:	20 gm
Glycerol	:	16 ml
K_2HPO_4	:	1.5 gm
$MgSO_4$	:	1.5 g
Agar	:	16 g
Distilled water	:	1000 ml

The collected soil samples were serially diluted using sterile saline solution. Initially the test tubes containing 9 ml sterile saline were labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. About 1g of the collected soil sample was weighed using electronic balance and added to the test tube labeled as 10^{-1}. From the first test tube, 1 ml of suspension was pipette out into the second test tube and different dilutions of working samples were prepared by serially diluting the solution up to $10^{-6.}$ Serially diluted soil samples were plated onto nutrient agar medium and King'B Agar medium by pour plate technique. The plates were incubated at room temperature for 24 hours. After incubation, the colonies obtained were picked and purified by repeated streaking on nutrient agar.

Gram staining a differential technique developed by Christain gram, used as a first step in identification of isolated bacteria before carrying out other tests. Gram staining differentiate bacteria into two distinctly separate groups called gram positive and gram negative by the chemical and physical properties of their cell wall

3.2. Screening of antagonistic potential of selected pigmented bacterial isolate

The antimicrobial potential of selected isolates against fungal pathogens was screened by dual culture technique (Ji et al., 2014). A fungal disc was taken from the test pathogen *Fusarium sp*. and placed 3 cm from the margin of the PDA plate. The antagonistic bacteria were streaked as a single straight line through one end of the nutrient agar plate opposite of the pathogen disc. The plates were incubated at room temperature for 7 days. The antagonistic effects of the isolates against fungal pathogens were monitored for the formation of inhibition zones starting from third day. Plates inoculated with selected pathogens in the absence of antagonist strains were also be maintained as negative controls. The assay will be replicated three times.

3.3. Molecular identification of selected pigmented rhizosphere bacteria

The selected pigmented rhizosphere bacterial isolate with the potential against plant pathogen were further subjected to molecular identification. For this, genomic DNA was isolated followed by PCR amplification and sequence analysis of 16 S rDNA. The sequence was further be subjected to BLAST analysis.

IV. RESULTS AND DISCUSSION

4.1. Isolation of rhizosphere bacteria

The bacterial colonies were isolated in Nutrient agar and Kings'B agar medium. After 24 hours of incubation in the room temperature the colonies were subjected to grow on the culture plates (Fig. 1). The majority of the colonies (almost 70%) obtained are white, creamy coloured and some are pigmented in nature. From among the various colonies formed after incubation, a purple pigmented colony named as RPB (Rhizosphere Purple Bacteria) were selected, purified and used for further studies (Fig. 2).

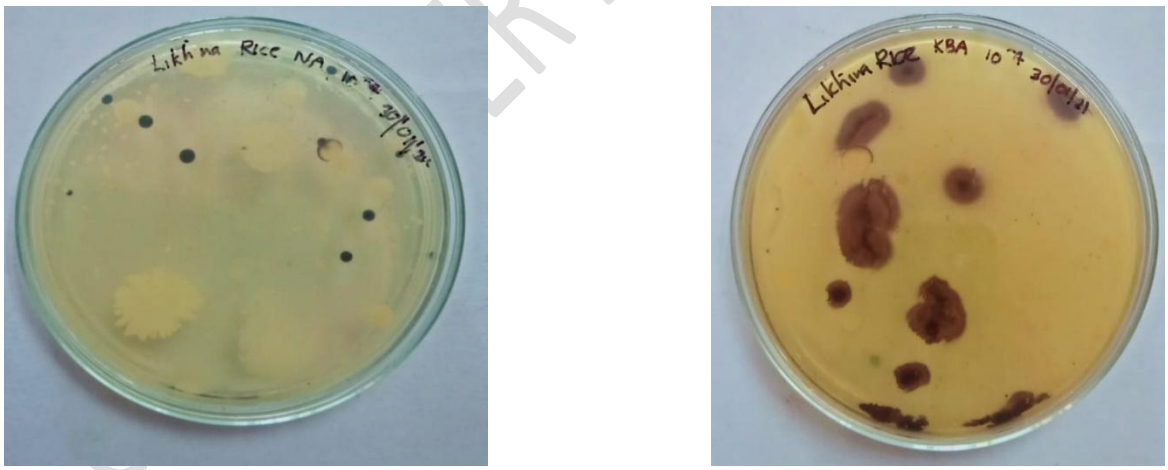

Fig. 1 Isolation of rhizosphere bacteria from soil

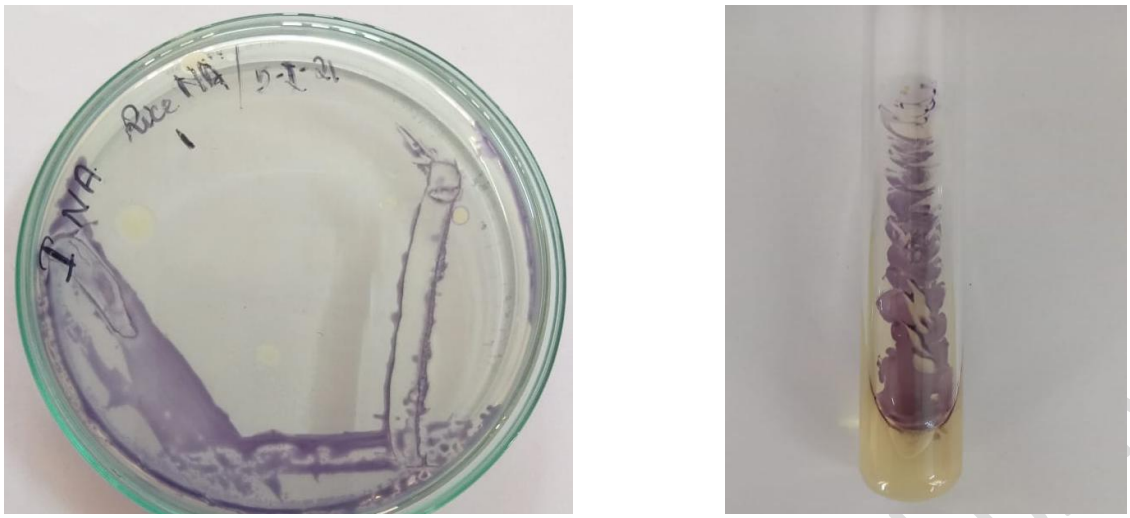

Fig. 2 Purification and Subculturing of RPB isolate.

4.2. Gram staining of selected pigmented bacterial isolates

The selected isolate was subjected to gram staining and observed under oil immersion objective. It was found that the purple pigmented bacteria was gram negative and rod shaped bacteria (Fig. 3).

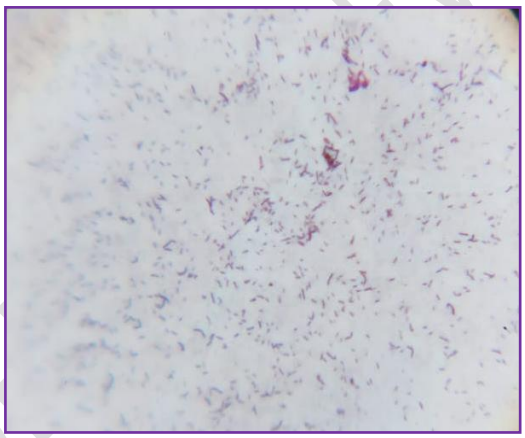

Fig. 3 Gram staining result of RPB

4.3. Screening of antagonistic potential of selected pigmented bacterial isolate

Dual culture technique revealed the antimicrobial potential of selected isolate against fungal pathogen. Thus the selected pigment isolate has antagonistic ability to produce non-volatile compounds to inhibit the test pathogen were further confirmed (Fig. 4). This property of bacteria helps the plants to prevent pathogenic affect of other microorganisms.

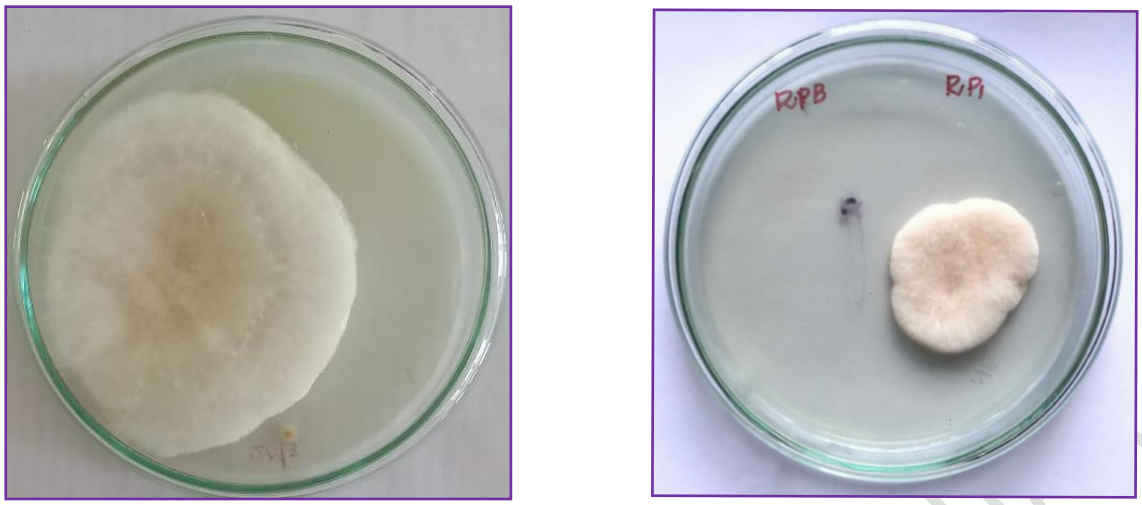

Fig. 4 Antagonistic effect of RPB against *Fusarium sp.* along with its control

4.4. Molecular identification of selected pigmented rhizosphere bacteria

Molecular identification of selected isolates was carried out by 16SrDNA sequence based method. Isolated genomic DNA was used as template for polymerase chain reaction (PCR).

4.5. BLAST analysis and phylogenetic analysis of 16SrDNA sequence

The sequence similarity of the obtained 16SrDNA sequence was analysed by comparing it with related sequences available in the National Center for Biotechnology information (NCBI) data. The 16SrDNA sequence data obtained were aligned using BioEdit programme and subjected to BLAST analysis (Zhang et al., 2000). The phylogenetic analysis of the 16S rDNA sequence of the isolates obtained in the study was also conducted with MEGA 6 using neighbor-joining method with 1,000 bootstrap replicates (Tamura et al., 2013). BLAST result of the selected isolate RPB showed 100% similarity with the available database sequence of *Chromobacterium violaceum.*

Nucleotide sequence of 16SRRNA Gene - *Chromobacterium violaceum*.

TAATGCGTCGGAATGTACCGTGTAATGGGGGATAGCTCGGCGAAAGCCGGATTAATACCGCATAC

GCCCTGAGGGGGAAGCGGGGGATCGAAAGACCTCGCGTTATACGAGCAGCCGACGTCTGATTAGC

TAGTTGGTGAGGTAAGAGCTCACCAAGGCGACGATCAGTAGCGGGTCTGAGAGGATGATCCGCCA

CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGG

GGCAACCCTGATCCAGCCATGCCGCGTGTCTGAAGAAGGCCTTCGGGTTGTAAAGGACTTTTGTCA

GGGAGGAAATCCCGCTGGTTAATACCCGGCGGGGATGACAGT ACCTGAAGAA

IV. SUMMARY AND CONCLUSION

Naturally occurring beneficial rhizobacteria were collectively called plant growth-promoting rhizobacteria (PGPR) which inhabit plant roots, plays a crucial role in setting for interactions between plant and microbes. This results disease free micro environment in soil leads to hazzle free growth of the plants. When food security and rural livelihood are top priorities, PGPR has drawn particular attention for its capacity to increase productivity, sustainability, and profitability in agriculture. The rhizobacteria isolated in this study , *Chromobacterium violaceum* is not normally seen in the rhizosphere region of paddy. Its ability to produce bioactive compounds with antifungal properties highlights its potential as an eco-friendly alternative to chemical

pesticides. The observed inhibitory effects against a range of pathogenic fungi suggest that Chromobacterium violaceum could play a significant role in sustainable agricultural practices, reducing crop losses by diseases and enhancing food security.This also saves the expenses incurred due to agriculture inputs and for sustainable, eco – friendly and economically feasible farming.

V. REFERENCES

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