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ISOLATION, DETECTION AND CHARACTERIZATION OF RHIZOBACTERIA IN RHIZOSPHERE SOIL OF PADDY (*ORYZA SATIVA*) THALAVADY, KERALA

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Abstract: In this study, the rice cultivating areas in Thalavady village, Alappuzha were surveyed for the isolation and screening of rhizospheric bacteria. The bacteria were isolated on Nutrient Agar and KBA medium. The pigmented colony was identified by Gram staining procedure and its antagonistic property was screened by dual culture method. Then 16SrDNA based identification was carried out and identified as *Serratia marcescens*.This bacteria is not commonlyfound in the rhizosphere soil. But in this study, it was isolated from the rhizosphere soil ofpaddy. To study its inhibitory effect towards *Fusarium* spp. Upon microscopic analysis, the pigment extract prepared from isolated *Serratia spp*.was found to cause remarkable distortion on fungal morphology. This was followed by analysis UV– Visible Spectrometry showing absorbance maxima at 535 nm that confirmed the prodigiosin pigment. Therefore, the broad antifungal activity of the prodigiosin-producing strain indicates its potential to be exploited as a natural ecofriendly biocontrol agent.

Index Terms **-Antagonistic, inhibitory, spectrometry, prodigiosin, Serratia, rhizosphere, rhizobacteria**

I. INTRODUCTION

Rice is an important crop plant which feeds over 50% of the global population. Rice, which is being cultivated for several years in the Indian sub continent, is just not a grain, it's a lifeline.Kerala has a rich history and tradition of rice cultivation. It occupies 7.46% of the total cropped area of the state. Rice rhizosphere contains a high diversity of plant growth promoting bacteria (PGPR).Generally, bacteria are the major population colonizing the rice rhizophere, which can exert positive, negative or neutral influences on plant growth.During seed germination and seedling growth, the developing plant interacts with a range of microorganisms present in the surrounding soil. As seeds germinate and roots grow through the soil, the release of organic material provides the driving force for the development of active microbial populations in this zone. This phenomenon is referred as the rhizosphere effect .Exploring these microorganisms by unraveling their possible relationships with plants has launched a new and fascinating area of investigations in the rhizosphere research. Thus, rhizosphere provides the frontline defense for plant roots against attack by soilborne pathogens.

PLANT GROWTH PROMOTING RHIZOBACTERIA

Plant growth promoting Rhizobacteria (PGPR) are a heterogeneous group of soil bacteria that colonize plant roots and enhance plant growth promotion activity through different mechanisms. These different mechanisms may be classified into direct and indirect mechanisms. PGPR affect plant growth directly by increasing nutrient cycling such as, biological nitrogen fixation (Ahmad

et al., 2008), siderophore production, solubilisation of phosphorus, synthesis of phytohormones or indirectly by synthesis of biocontrol compounds to inhibit phytopathogens.

The present work was designed to recover the microbes which should bear the inherent ability to outcompete the soil borne pathogens for survival in the natural environment.

II. NEED OF THE STUDY

Increasing incidence of Neuro-genital and carcinogenic diseases in Kuttanad region, especially those who are directly involved in agriculture. In addition to that decreasing trend on Rice production in recent years also pointing towards the depletion of beneficial micro fauna which plays a pivotal role in maintaining the crop production. Plant protection through naturally occurring free living beneficial strains are an ideal alternative choice for synthetic fertilizers and pesticides.

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III. RESEARCH METHODOLOGY

The methodology section outline the plan and method that how the study is conducted. This includes collection of soil samples, preparation of mediums, isolation of rhizosphere bacteria, Gram staining, screening of selected bacterial pigment, molecular identification. The details are as follows.

1. Collection of Sample

The soil samples were collected from fields cultivated with paddy (Oryza sativa) from thalavady village in kuttanad, Alappuzha. Two samples were collected in sterile plastic bags for the analysis purpose. Of the two samples, one sample is collected along with intact root system of paddy which is the rhizosphere soil and the second sample was collected from the area near to the rhizosphere.About250gofsoilalongwiththeintactrootsystemiscollectedinplasticbags and tied them tightly and each sample systematically labeled with date, place, sample , number.

2. PREPARATION OF MEDIUM

A. NUTRIENTAGAR

For solid media preparation

Peptone powder	: 5g
Beef extract	: 3g
Nacl	: 5g
Agar	: 15g
Distilledwater	: 1000ml

B. KINGS B AGAR MEDIUM

For solid media preparation

Peptonepowder	:20 g
Glycerol	:16 ml
K2HPO4	:1.5 g
MgSO4	:1.5 g
Agar	:16 g
Distilled water	:1000 ml

C. POTATO DEXTROSE AGAR MEDIUM

Potato extract	: 4gm
Dextrose	: 20gm
Agar-agar	:50gm
Distilled water	: 1000

3. ISOLATION OF RHIZOSPHERE BACTERIA

The collected soil samples were serially diluted using sterile saline solution. Isolation of soil microbes is done via pour plate method in nutrient agar medium for bacteria, King's B medium for Pseudomonas species and Potato dextrose agar medium for the isolation of fungi. The isolation procedure is done through various steps. The soil is the important medium for microbial growth. So even a milligram of soil contain uncountable bulk load of microorganisms. Therefore, soil microorganisms were isolated by serial dilution technique on nutrient agar medium (NAM). One gram of soil from sample was separately suspended in 10 ml of distilled water and mixed well for 15 minutes and shake well. Each suspension was serially diluted from 10^{-1}-to 10^{-5}. Thus the microbial load comes to a countable range.
Pour plate technique was carried out to isolate the organism from the diluted sample. For this molted medium was transferred to 1 ml of the sample in the Petri plate. Then the plates are incubated at room temperature for 24 – 48 hours. The most prominent colonies were isolated and maintained at 4°C for further studies. The isolated Colony of interest was purified by streaking techniques and the culture is maintained in slant .

4.GRAM STAINING OF SELECTED BACTERIAL ISOLATE

Gram staining is used as a first step in identification of isolated bacteria before carrying out other tests. It is the differential staining technique developed by Christian Gram, in 1884 to differentiate bacteria that are morphologically indistinguishable. Gram staining serves to differentiate bacteria into two distinctly separate groups called Gram positive and Gram negative.

5. 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 took from the test pathogen (*Fusarium* .sp). and placed 3cm from the margins of the PDA plate. The antagonistic bacteria were streaked as a single straight line through one end of the nutrient agar plate opposite to the pathogen disc. The plates were incubated at room temperature for 7days. The antagonistic effect of the isolates against fungal pathogens was monitored. It is seen that inhibition zone starts to form from third day itself. Plates inoculated with selected pathogens in the absence of antagonist strains were also be maintained as negative controls. This assay was replicated for three times.

6. MOLECULAR IDENTIFICATION OF SELECTED PIGMENTED RHIZOSPHERE BACTERIA

The selected pigmented rhizosphere bacteria 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 16S rDNA. The sequence was further subjected to BLAST analysis and related species were analyzed for final identification of the bacterial strain.

IV. RESULTS AND DISCUSSION

A. Isolation of rhizosphere bacteria

The bacterial colonies were isolated on Nutrient Agar media and King's B Agar media. After 24 hours of incubation, the colonies grow on the plates. Majority of the isolated colonies were creamy colored (about 70%) and some were pigmented. Most of the colonies were round, branched or rod shaped. A red pigmented colony named RRB (Rhizosphere red bacteria) were selected, purified and used for further studies and others were discarded.

B. Gram staining of selected bacterial isolate

The selected pigmented bacterial isolate was identified by Gram staining prior to the further studies and this showed that the pigmented

Bacterial is Gram negative rod.

C. Screening of antagonistic potential of selected pigmented bacterial isolate

The antimicrobial potential of selected isolate against fungal pathogens was screened by dual culture technique. A fungal disc of the test Pathogen was placed on PDA plate and the antagonistic bacteria were streaked. The plates were incubated for 7 days. The bacteria inhibits the growth of fungi, whereas, fungal disc in the control plate shows vigorous growth. Hence, it is concluded that the selected pigmented Isolate has antimicrobial activity. The secretion from the bacteria protects the plant from the pathogenic fungi.

Fig.1.Antagonisticeffectof RRB against Fusarium sp.along its control

D. Molecular identification of selected pigmented bacteria

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

E. BLAST analysis and phylogenetic analysis of 16SrDNA sequence

The sequence similarity of the obtained 16SrDNA sequence was analyzed by comparing it with related sequences available in the National Center for Biotechnology information (NCBI) data. The 16SrDNA sequence data obtained were aligned using BioEditprogramme 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 RRB showed 100% similarity with the available database sequence of *Serratia marcescens.*

NUCLEOTIDESEQUENCEOF16SRRNA GENE

<SR2148-RRB-RSF1_C01.ab1

GCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACT
GGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGG
CCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCA
CCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAG
ACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA
AGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTT
CAGCGAGGAGGAAGGTGGTGAACTTAATACGTTCATCAATTGACGTTACTCGCAGA
AGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGT
TAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGA
AATCCCCGGGCTCAACCTGGGAACTGCATTTGAAAACTGGCAAGCTAGAGTCTCGTA
GAGGGGGGTAGAATTCC

Showing 100%similarityto *Serratia marcescens.*

Current soil management strategies are mainly depending on inorganic chemical based fertilizers, which caused a serious threat to human health and environment. Plant growth promoting rhizobacteria(PGPR) are naturally occurring soil bacteria that aggressively Colonize plant roots and benefit plants by providing growth promotion. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoot growth, seedling germination, plant health vigor, height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content. Therefore, the broad antifungal activity of the *Serratia marcescens* strain indicates its potential to be exploited as a natural ecofriendly biocontrol agent. Moreover, *Serratia marcesecns* is not generally present in rhizosphere soil but in this experiment it is isolated from rhizosphere soil from kuttanad.

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VI. REFERENCES

- A.K Shrivastava, KhilendraDewangan and D.K Shrivastava.(2014)Plant growth promoting rhizobacterial strains from rice rhizospheric soil. 3-5
- Ajay Kumar, Amit Kumar, Shikha Devi, Sandip Patil, ChandaniPayal and Sushila Negi. (2012,)Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study,1-3
- Besler KR, Little EL: Diversity ofSerratia marcescensStrains Associated with Cucurbit YellowVineDiseaseinGeorgia. PlantDis.(2017);101(1):129–136.PubMedAbstract |Publisher Full Text
- Beveridge, T. J. (2001). "Use of the Gram stain in microbiology". Biotechnic &Histochemistry. 76 (3): 111–118.
- BrigidaPimentelVillardeQueiroz;ItamarSoaresdeMelo.(2006)AntagonismofSerratia marcescens towards *Phytophthora parasitica*and its effects in promoting the growth of citrus.vol 37, 2-4.
- Brooks, D. S., C. F. Gonzalez, D. N. Apple, and T. H. Filer. (1994). Evaluation ofendophyticbacteriaaspotentialbiologicalcontrolagentsforoak wilt.Biol. Control4:373- 381
- DavidR.Caprette,RiceUniversity.(2012)Enteriobacteriaceae:Serratiamarcescens,1-3.
- de Souza, R., A. Beneduzi, A. Ambrosini, P.B. da Costa, J. Meyer, L.K. Vargas, R. Schoenfeld and L.M.P. Passaglia. (2012). The effect of plant growth-promoting rhizobacteria on the growth of rice (Oryza sativa L.) cropped in southern Brazilian fields. Plant Soil. 366: 585–603. doi 10.1007/s11104-012-1430-1.
- Dhar Purkayastha G, Mangar P, Saha A, SahaD .(2018) Evaluation of the biocontrol efficacyofaSerratiamarcescensstrainindigenous totearhizosphereforthemanagement of root rot disease in tea ,1-2
- Jaiganesh V., A. Eswaran, P. Balabaskar, C. Kannan [2007], Antagonistic activity of Serratia marcescens against Pyricularia oryzae,1-2
- Kumar, A., B.R. Maurya, and R. Raghuwansh. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (Triticum aestivum L.). Biocatalysis and Agricultural Biotechnology 3: 121–128.
- Palacio-CastañedaValentin,Pérez-HoyosAlejandra,Carrascal-CorreaDaniel,Osorio- EcheverriVíctor Manuel vol.21(2019). Antibacterial pigment production by Serratia marcescens using different casein types obtained from milk. 3-4.
- Soytong, K.1 ,Unthuraloet, K.1 and Younes RezaeeDanesh2.(2020)Serratia marcescens KMITL2020 as a plant growth stimulant and bacterial antagonist to control brown leaf spot of rice caused by Drechsleraoryzae ,vol 16,1
- Vijayalakshmi, K.1 and Jagathy. K2.(2016) Production of Prodigiosin from Serratia marcescens and its antioxidant and anticancer potential. Volume 3, 75-87.