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Biocontrol potential of *Chromobacterium violaceum* isolated from the below-Sea-Level Paddy Fields of Kuttanad, Alappuzha, Kerala, India

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Abstract
Rice, the edible starchy cereal grain is the most important food crop grown in Kerala especially in Palakkad and Alappuzha, Kerala, India. However, the occurrence of rice diseases during cultivation poses a significant challenge to achieving optimal yields. Among the major pathogens, Pythium species, which cause seedling blight or root rot are of important concern, particularly in water-seeded rice production. *Pythium myriotylum* is a serious threat to rice production, causing significant damage to seedlings and reducing yields. Plant growth-promoting rhizobacteria act as an inhibitory factor for various pathogenic microorganisms through the production of some metabolites which stops the further growth of the particular pathogen. Thus the present study involved the isolation of rhizosphere bacteria, molecular identification and screening of their antagonistic properties against plant pathogen *Pythium myriotylum*. It was come to found that the selected pigmented bacteria have some antagonistic properties, which completely inhibited the further growth of the test pathogen. Furthermore, they were identified as potent phosphate solubilizers. For this, genomic DNA was isolated followed by PCR amplification and sequence analysis of 16S rDNA. The sequence were further be subjected to BLAST analysis and showed 100% similarity with the available database sequence of *Chromobacterium violaceum*. Therefore rhizosphere bacteria isolated in the current work indicates their potential to be exploited serve effectively as both a biofertilizer and a prominent biocontrol agent against soil-borne pathogens.

Keywords: Phytopathogen, *Pythium myriotylum*, *Chromobacterium violaceum*, Antifungal acivity, Biofertilizer, Phosphate solubilizer

Introduction

Rice is considered as one of the most important crop plants in the world and rice diseases represent a primary constraint on productivity, significantly limiting the potential for increased global yields. Numerous reports indicate that diseases not only suppress yield but also significantly degrade grain quality. To sustain and enhance both productivity and crop standards, it is essential to protect rice from the pathogens responsible for both major and emerging diseases [1]. Kuttanad and Palakkad serve as Kerala's primary rice-growing hubs. Together with Thrissur and Kottayam, these districts accounted for 82.5% of the state's total rice production that year. Notably, the Kuttanad below sea-level farming system (KBSFS) is a unique marvel, it is the only system in India where rice is cultivated below sea level. This wetland ecosystem, historically revered as 'Kerala's Rice Bowl,' extending through the districts of Alappuzha, Kottayam and Pathanamthitta [2].

While rice is a global staple, cultivation is frequently hindered by diseases that threaten overall yields. Of particular concern are *Pythium* species, ubiquitous soil-borne oomycetes that range from opportunistic to highly virulent. These pathogens primarily target juvenile tissues, causing pre- and post-emergence damping-off, though they can also infect mature roots to cause severe necrosis and stunting. Despite the identification of numerous disease-resistance genes in rice, research into the specific mechanisms of *Pythium* resistance remains remarkably limited [3]. Thus understanding the etiology, symptomatology, and management of these pathogens is essential for mitigating their impact.

Modern rice cultivation frequently depends on chemical fertilizers and pesticides to maximize yields. However, excessive application often triggers nutrient runoff, leading to the eutrophication of downstream ecosystems. In Kerala, this intensive chemical use has specifically resulted in water pollution and a decline in local biodiversity. This indicates the significance of sustainable and organic methods which are economical and have healthier outcome when compared to chemicals. The rhizosphere microbes exhibit several antagonistic properties as well as a wide variety of ecological niche and a high diversity of soil microorganisms. Plant growth-promoting rhizobacteria or 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.

Chromobacterium violaceum is a Gram-negative β-proteobacterium prevalent in tropical and subtropical soil and aquatic environments. Typical of free-living microbes, its versatile metabolism allows it to adapt to fluctuating environmental conditions. Beyond its adaptability, this bacterium synthesizes various compounds with applications in environmental detoxification, bioprospecting, pest control, and medicine. A notable example is violacein, a pigment characterized by its potent cytotoxic and antibacterial properties [4, 5]. Hence the current s study mainly aims to identify the antifungal potential of a rhizobacteria *Chromobacterium violaceum* isolated from the paddy fields of Kuttanadu against prominent rice pathogen *Pythium myriotylum* and screened for its potential to solubilize phosphate that highlights the potential of our selected strain as an effective biofertilizer to establishes a sustainable pathway for resilient rice production and improved soil health.

Materials and Methods

Sampling and Isolation of rhizosphere bacteria

The soil samples were collected in sterile plastic bags from the fields cultivated with paddy (*Oryza sativa* L.*)* from different Kuttanadu regions like Shastangal and Pallathuruthy. Approximately 25 g of soil, including the intact root system, was collected in tightly sealed plastic bags. Each sample was labelled with the date, location, sample number, and type. Following collection, samples underwent serial dilution and were plated on nutrient agar, then incubated at room temperature for 24 hours. Resulting colonies were isolated and purified via repeated streaking [6]. Finally, pure isolates were screened for both antifungal and phosphate solubilizing potential.

Collection of fungal plant pathogen

The plant pathogenic fungi *Pythium myriotylum* was collected from the Research Department of School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India. The fungal isolate was used for evaluating the antagonistic potential of selected bacterial isolate. The fungal morphology was studied by observing the colony features (colour, shape, size and hyphae) and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with small portion of mycelium [7].

Screening of antagonistic potential of selected bacterial isolate

The prominent bacterial isolate (SRB 1) with purple colour pigment was selected and screened for its antifungal activity against *Pythium myriotylum* using an *in vitro* dual culture assay. A fungal disc from the test pathogen was placed 3 cm from the margin of a Potato Dextrose Agar (PDA) plate. On the opposite side, the selected bacterial isolates were streaked in a single straight line. The plates were incubated at room temperature for seven days, with antagonistic activity monitored by the appearance of inhibition zones starting from the third day. Plates inoculated with selected fungal pathogens in the absence of antagonist bacterial strains were also maintained as negative controls. All assays were performed in triplicate [8, 9].

Screening for Phosphate Solubilizing Potential

The selected bacterial isolate was evaluated for its ability to solubilize inorganic phosphorus. Isolate was inoculated onto Pikovskaya's agar (PKA) medium (pH 7) containing 5 g/L of tri-calcium phosphate as the sole phosphorus source. Following 48 hours of incubation at 28°C, colonies exhibiting distinct halophilic zones were identified as potent phosphate solubilizers [10, 11]. To quantify this potential, the diameters of both the clear zones and the colonies were measured to calculate the Phosphate Solubilization Index (PSI) using the following formula:

$$\text{Phosphate Solubilization Index (SI)} = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

Molecular identification of pigmented bacterial isolate

The pigmented rhizosphere bacterial isolate (SRB 1) with prominent antifungal activity against fungal pathogen *Pythium myriotylum* was subjected to molecular identification. Molecular identification was performed via 16S rDNA sequencing, using total genomic DNA as the PCR template. For this, the total genomic DNA was isolated from the selected strain. This was used as a template for polymerase chain reaction. These PCR products were sequenced at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, Kerala, India. This was followed by the alignment of 16SrDNA sequence data obtained using BioEdit programme and subjected to BLAST analysis as per previous reports [12, 13].

Results

Isolation and identification of rhizosphere bacteria SRB1

Rhizospheric soil samples, comprising various proportions of sand and clay, were collected from vigorous paddy plants in the Kuttanad region. From the purified isolates, a morphologically distinct, prominent purple pigmented colony (SRB 1) was selected and purified for further characterization (Fig.1). Molecular identification was performed using 16S rDNA sequencing. The resulting sequence was aligned via BioEdit and compared with the NCBI database using BLAST [14]. Phylogenetic analysis was subsequently conducted in MEGA 6 using the neighbor-joining method with 1,000 bootstrap replicates [15]. BLAST result of the selected isolate SRB-1 showed 100% similarity with the available database sequence of *Chromobacterium violaceum*. The sequences were deposited in GenBank and the accession number OK301341.1 was obtained.

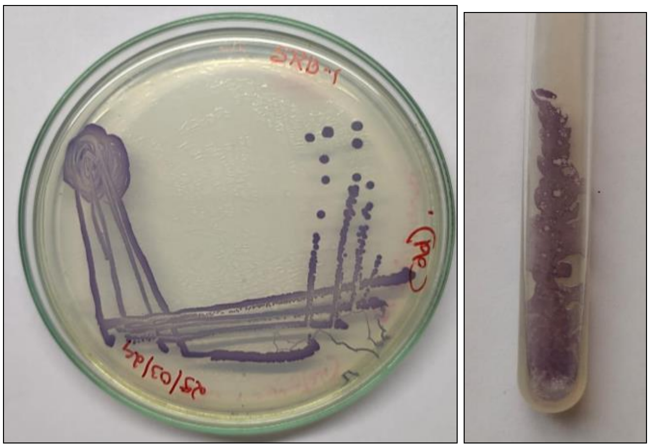

Fig 1: Pure culture of rhizosphere bacterial isolate SRB 1

Morphological characterization of *Pythium myriotylum*

The Pythium *myriotylum* isolate were observed to have aseptate hyphae with white cottony growth on Potato Dextrose Agar plates. Distinctive features of Pythium: oogonial wall, oospores, antheridia and sporangia were observed in purified isolate. *Pythium myriotylum* is a destructive oomycete pathogen that poses a severe threat to water-seeded rice production. By targeting succulent root tips and germinating seedlings, it causes damping-off and root rot, leading to poor crop establishment and substantial yield reduction. In addition, the encysted zoospores, oospores and sporangia of this fungi can persist in soil for long periods [16, 7]. As this is one of the prevalent diseases impacting rice production in Kerala, identifying its fungicide sensitivity is essential for developing prominent management strategies.

Biocontrol efficacy of isolated rhizobacteria against *Pythium myriotylum*

To identify strains exhibiting potent antifungal activity against phytopathogen, the antagonistic activity of the pigmented rhizobacterium *Chromobacterium violaceum* (SRB 1) was evaluated against the *Pythium myriotylum* using the dual-culture technique on Potato Dextrose Agar. The results demonstrated that *C. violaceum* significantly inhibited the mycelial growth of *P. myriotylum* (Fig. 2), producing a distinct zone of inhibition indicative of antimicrobial metabolites. In contrast, the hyphae in the control plates remained normal and intact. These findings align with [17], who reported that bio-products synthesized by the many bacterial species exhibit potent inhibitory activity against various fungal phytopathogens. Furthermore, the broad-spectrum antagonism observed in *C. violaceum* is often attributed to the production of specialized compounds that cause structural distortion of fungal mycelia.

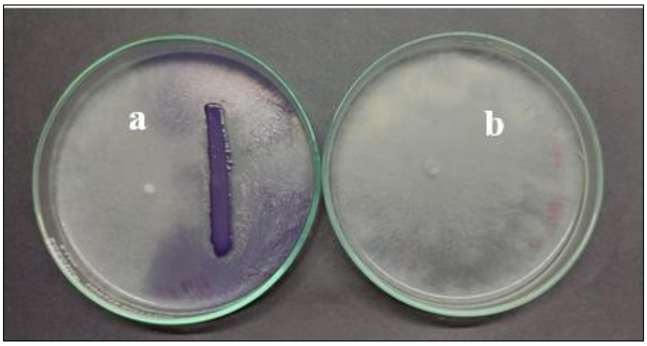

Fig 2: Mycolytic effect of *C. violaceum* against *Pythium myriotylum*. (a) Test Plate (b) Control plate

Phosphate Solubilization Potential of *Chromobacterium violaceumS*

The SRB 1 isolate, identified as *Chromobacterium violaceum*, exhibited significant inorganic phosphate solubilization on PKA medium. This activity was visualized by an excellent halo zone (Fig 3), yielding a Solubilization Index (SI) of 2.4 after 24 hours of incubation.

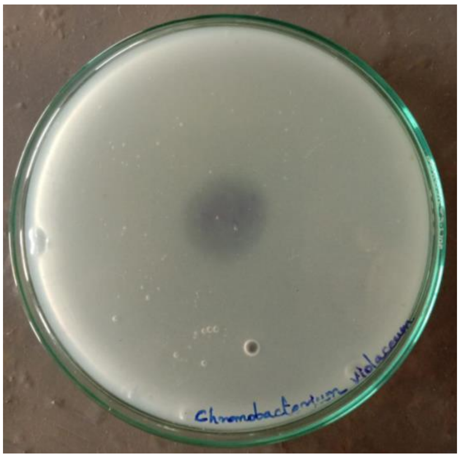

Fig 3: Phosphate solubilizing efficiency of *Chromobacterium violaceum*

Discussion

As a primary staple for over half the global population, rice is central to the history, nutrition, and economies of billions. Beyond food security, rice ecosystems provide critical ecological services essential to human well-being. In addition, Rice cultivation is foundational to Kerala's socio-economic structure, exerting a profound influence on the state's agricultural landscape and the daily livelihoods of its inhabitants. Ensuring the continuity of these benefits requires the implementation of effective management strategies [18].

As the global population increases, the demand of the population for food is increasing day by day. While researchers continuously develop high-yield crop varieties these often susceptible to evolving pathogens within a few years of introduction. Beyond crop loss, microbial contamination significantly degrades the nutritional and commercial value of food products [19]. It was reported that major soil-borne pathogens, including species of *Pythium*, *Fusarium*, *Rhizoctonia*, *Phytophthora*, *Botrytis*, *Penicillium*, *Alternaria*, *Aspergillus*, *Ascochyta*, and *Colletotrichum*, target host plants at various developmental stages [20]. To mitigate these threats, biological control offers a sustainable alternative to chemical interventions, providing a cost-effective and eco-friendly strategy for pathogen management.

Fungal pathogens cause severe infections in cereal crops, fruits and vegetables, globally, posing a significant threat to agricultural productivity. Biological control serves as a sustainable alternative to synthetic fungicide, utilizing a diverse array of microorganisms including bacteria, fungi, and nematodes as biocontrol agents. These agents do not merely suppress the growth of target pathogens; they actively promote plant health by facilitating nutrient uptake through mutualistic associations. Furthermore, the implementation of BCAs is cost-effective and environmentally secure, as they do not leave harmful residues in water, soil, or plant products [21].

Plant growth-promoting rhizobacteria are prominent inhabitants of the rhizosphere, playing a vital role in enhancing soil fertility and stimulating plant growth. Beyond growth promotion, PGPR confer resilience against abiotic stressors, including heavy metal toxicity, salinity, drought, and nutrient deficiencies. These bacteria also fortify plant health to combat various diseases [22] through two primary antagonistic modes of action: direct and indirect. In the direct mode, PGPR suppress pathogens through mechanisms such as hyperparasitism or the secretion of lytic enzymes. Conversely, indirect antagonism involves the induction of systemic resistance (ISR) within the host plant or the production of antibiotic substances that inhibit pathogenic growth [23]. These antimicrobial secondary metabolites, typically low-molecular-weight compounds have proven lethal to a wide range of phytopathogenic microbes [24]. Hence utilizing microorganisms as biocontrol agents offers an ecologically sustainable and cost-effective alternative to traditional methods, positioning *C. violaceum* as a prime candidate for such innovation [25]. Despite its pharmaceutical fame, the biotechnological application of *C. violaceum* in solving agricultural challenges remains largely unexplored. It was also suggested that a synergistic mechanism in which the extracellular chitinase Chi54 and the novel antibiotic chromobactomycin operate in tandem to drive the suppressive activity of *Chromobacterium* sp. strain C61 against plant pathogens [26].

Phosphorus (P) is a fundamental soil macronutrient, occupying a critical role in plant development and metabolic processes. To overcome phosphorus limitations in the soil, phosphate-solubilizing microorganisms (PSMs) modulate plant metabolism by significantly enhancing the bioavailability of soluble phosphorus [27]. PSMs facilitate the solubilization of insoluble phosphates by producing organic acids like gluconic, oxalic, and succinic and also by the production of the production of H^+ [28]. These biochemical interventions enhance mineral solubility, directly increasing the phosphorus bioavailability required for optimal plant growth). In addition, at the molecular level, PSMs trigger signaling pathways and up regulate stress-responsive endogenous genes, enabling plants to better tolerate environmental extremes such as salinity, drought, and heavy metal toxicity [29].

Utilizing rhizospheric bacteria as biocontrol agents offers a viable pathway to enhance soil fertility, plant health, and overall crop productivity. Hence the findings of the current study clearly demonstrate that the isolate *Chromobacterium violaceum* serves as an effective biocontrol agent against the fungal pathogen, *Pythium myriotylum*. This pathogen severely impacts food security by extensive damage to rice seedlings by inducing root rot, seed decay, and both pre- and post-emergence damping-off. These infections significantly diminish seedling survival rates and result in poor stand establishment. In addition, this fungal pathogen is particularly destructive in warm, high-moisture, and poorly drained environments, where it aggressively invades succulent young root tissues.

Conclusion

Modern agricultural practices remain heavily dependent on synthetic fertilizers and pesticides; however, the intensive application of these chemicals has resulted in substantial environmental degradation, including human health hazards, the disruption of natural nutrient cycling, and the erosion of microbial biodiversity. These consequences underscore the urgent need for sustainable, organic alternatives that are both economical and ecologically benign. Thus, the *Chromobacterium violaceum* isolate characterized in this work, derived from the paddy rhizosphere of Kuttanadu region is expected to exhibit robust root colonization and long-term persistence in the field, functioning as both a potent biofertilizer and a prominent biocontrol agent.

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Statements and Declarations

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. On behalf of all authors, the corresponding author states that there is no conflict of interest.

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