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PGPR: RECENT CHARACTERIZATION AND BENEFICIAL EFFECT AGAINST FUNGAL DISEASES OF CHILLI

[1]Uttam Bouri*, [1]Joseph Soren and [2]Subhas Patar

Email

uttambouri757@gmail.com

[1]Department of Plant Pathology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal- 741252, India

[2]Department of Biological Science, Midnapore City College (Affiliated by Vidyasagar University), Paschim Medinipur, West Bengal- 721129, India

Chilli (*Capsicum annum* L.) is a remunerative vegetable and spice-cum-cash crop of the Indian subcontinent. Chilli is also called red pepper which belongs to the family Solanaceae and genus *Capsicum* with chromosome number 2n = 24. It is believed to have originated in tropical America (Bosland, 1996). It is being extensively grown in regions with warm temperate, tropical and subtropical climates worldwide. Chilli produces two distinctive chemical compound groups namely capsaicinoids and carotenoids. Among these compounds, the abundant carotenoids not only contribute to its vibrant colour but also enhance its nutritional value (Perez-Galvez *et al.*, 2003). This versatile crop is recognized for its high content of essential vitamins such as C, A and B. Indian chilli has asserted its dominance in the global chilli market. Indian chilli is renowned on the global stage for its exceptional commercial attributes including its vibrant colour and distinctive pungency (Anonymous, 2021). India is the largest producer with 1.98 million tonnes and contributes 43% of world chilli production, followed by China, Ethiopia, Thailand, Pakistan and Bangladesh. In our country green chilli is cultivated in an area of 418.00 thousand ha. with an annual production of 4505.00 thousand MT and dried chilli is cultivated over an area of 852.00 thousand ha. with a production of 1578.00 thousand MT in 2021-22 (Anonymous, 2022). In India, major chilli-producing states are Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh, Rajasthan and West Bengal (Sahitya *et al.*, 2014).

Soil microorganisms, comprising a diverse array of organisms, have gained significant attention for their role in promoting plant growth and defending against soil-borne plant pathogens. Particularly, the beneficial free-living soil bacteria are commonly known as Plant Growth Promoting Rhizobacteria (PGPR). These PGPRs confer various advantages,

including the stimulation of growth-promoting hormones, phosphorus solubilization, production of siderophores and antibiotics, inhibition of plant ethylene production, and the induction of systemic resistance in plants against pathogens (Datta *et al.*, 2010). The direct interaction of these bacteria with root tissues, through root colonization, is deemed crucial for the promotion of plant growth. Employing biological control strategies with microbes, such as rhizobia, emerges as an alternative approach for disease management (PGPRs). Rhizobacteria function as antagonistic agents, reducing disease incidence and enhancing overall crop health. Consequently, addressing the escalating demand for chemical residue-free agricultural products necessitates the exploration of more effective and efficient bio-control agents (Kashyap *et al.*, 2020).

Isolation of PGPR Strains

Chilli plant seedlings, aged 5 to 6 weeks post-seeding were collected and then carefully removed from the soil. Soil samples from the immediate root zone known as the rhizosphere, were gathered. Using the serial dilution method, rhizobacterial strains were isolated from the collected soil samples and cultured on various growth media including TSA (Tryptone soya agar), NA (Nutrient Agar), CPG (Casamino peptone glucose) and Kings' B media. The plates were then incubated at 27±1°C for 48-72 hours. Bacteria exhibiting irregular and creamy white morphologies were selectively isolated and maintained on the YGCA (yeast glucose carbonate agar) medium (Kashyap *et al.*, 2020). These cultured strains are to be securely stored at 4ºC for future use in the isolation of rhizobacterial strains.

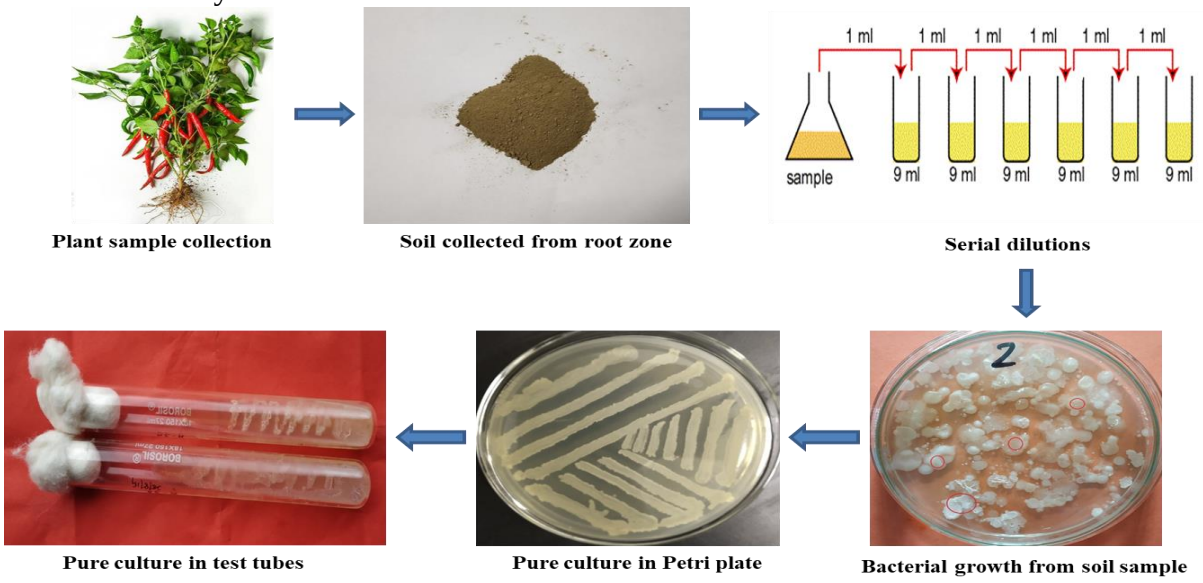


Fig. 1: Representative photographs of isolation of rhizobacteria

Morphological and Biochemical Characterization

The isolated bacterial strains underwent characterization based on morphological, cultural, staining and biochemical properties. Morphological characteristics, including colony colour, elevation, edge features and the presence or absence of capsules were assessed for each isolate. The gram nature of each strain was initially identified using crystal violet and safranin stain.

For biochemical characterization, standard microbiological techniques were employed to assess properties such as amylase, catalase, gelatin hydrolysis, citrate utilization, H_2S production, methyl red and urease. Additionally, a carbohydrate utilization test was conducted by inoculating isolated bacteria into a synthetic medium broth containing various carbohydrates like arabinose, cellobiose, dextrose, fructose, galactose, inositol, lactose, mannitol, maltose, raffinose and sucrose. The incubation period was at 27±1°C for 48-72 hours, during which the growth was measured at 600 nm (Datta *et al.,* 2010).

Characterization of Antagonism of Rhizobacteria Against Diseases of Chilli

The antagonistic potential of rhizobacterial isolates against chilli diseases was assessed using the dual culture method. The experiment involved replicating isolated chilli diseases on Potato Dextrose Agar (PDA). An actively growing pathogen mycelial plug was centrally placed on the agar medium with the PGPR strain streaked 2 cm away on both sides of the mycelial plug. The plates were subsequently incubated at 27±1°C for approximately 5 days or until the fungus leading edge in the control plate extended to the plate's edge (Dinesh, *et al.,* 2015). The radial growth of fungal mycelium was measured along with the percent inhibition of growth over untreated control and the percentage inhibition was compared with control using the formula:

$$I = \frac{C - T}{C} \times 100$$

Where,

I= Per cent growth inhibition, C = Colony diameter of pathogen in control, T = Colony diameter/radial growth of pathogen in treatment. Rhizobacterial isolates showed more than 50% inhibition against chilli pathogens.

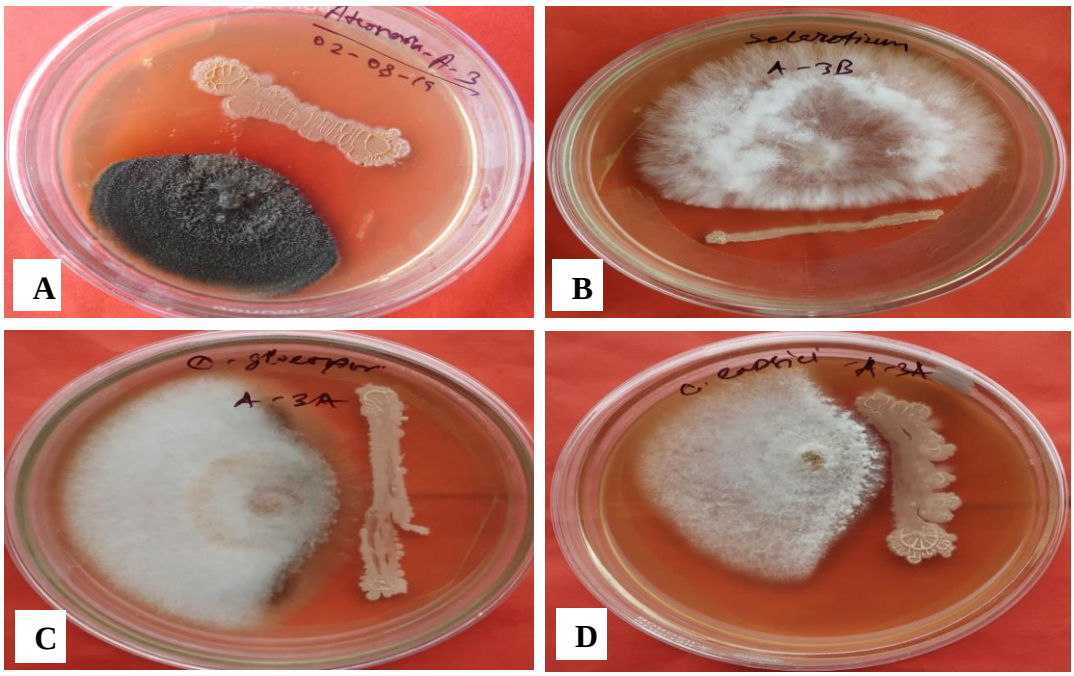
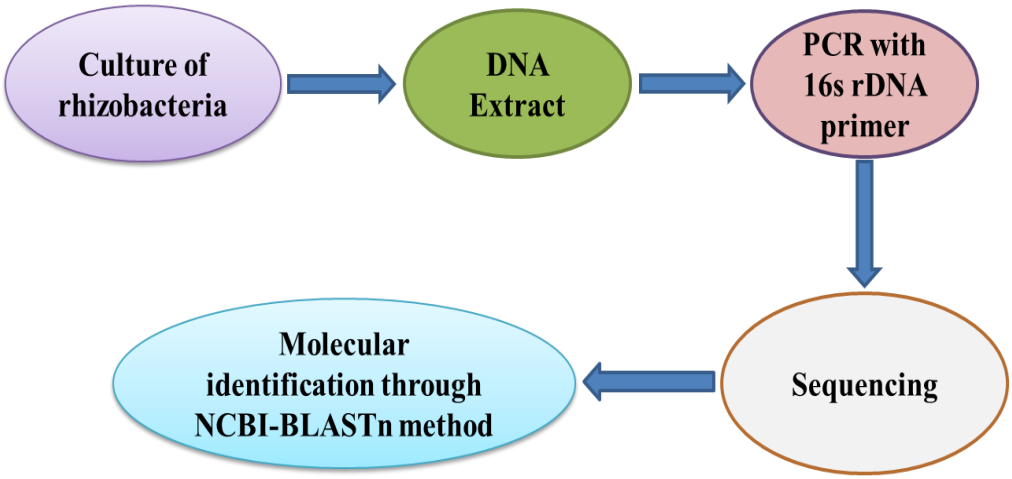


Fig. 2: Antagonistic potentiality of rhizobacteria against major fungal disease chilli. A] *Alternaria solani*, B] *S. rolfsii*, C] *Colletotrichum gloeosporioides* and D] *C. capsici*

Molecular Identification



Characterization of Rhizobacteria Isolates for Plant Growth-Promoting Traits

Indole acetic acid (IAA) production test:

To assess the production of indole-3-acetic acid (IAA) by bacterial isolates, 25 ml of rhizobacteria supernatant was collected. The pH of this solution was adjusted to 2.5 using 1N HCL in a 100 ml conical flask. Following this, an equal volume of diethyl ether was added

and left to incubate in darkness for 4 hours. The extraction of indole acetic acid was carried out at 4°C using diethyl ether in a separate funnel. The organic phase was discarded, and the solvent was combined. The determination of IAA in the methanol extract was performed by mixing 0.5 ml of the extract with 1.5 ml of double distilled water and 4 ml of sampler reagent (1 ml of 0.5M FeCl3 in 50 ml of 35% perchloric acid). This mixture was then incubated in darkness for 1 hour. The presence of IAA was indicated by the development of a pink-red colour and the absorbance was measured at 530 nm using a spectrophotometer.

Siderophore production test:

The assessment of siderophore production involved the examination of rhizobacterial isolates on CAS-agar Petri dishes. Utilizing sterile toothpicks, 72-hour-old cultures were introduced to CAS-agar plates through stabbing, followed by a two-week incubation at 28°C in the absence of light. Colonies exhibiting orange zones were identified as possessing siderophore activity. Control plates containing CAS-agar but remaining uninoculated underwent identical incubation conditions, revealing no observable colour change in CAS-blue agar during the 1-15 day incubation period.

Phosphate solubilization test:

The phosphate solubilization test utilizing rhizobacteria was done using Pikovskaya's agar medium. Cultures of the most promising antagonistic isolates from rhizobacterial samples aged 48 hours, were inoculated into Pikovskaya's (PVK) broth medium and subsequently incubated at 27±1°C for 3-5 days. During the incubation period, 1 ml of each bacterial culture was transferred to separate culture tubes and 10 ml of ammonium molybdate was added to each culture ensuring thorough mixing. The solution's blue colour intensity was then measured at 600 nm using a spectrophotometer and the corresponding amount of soluble phosphorus was determined by a standard curve.

Production of Hydrogen Cyanide (HCN) test:

Rhizobacteria isolated specifically for hydrogen cyanide (HCN) production, were streaked onto a nutrient agar medium supplemented with glycine (4.4 g/L). A Whatman number 1 filter paper, was soaked in a specific solution (0.5% picric acid and 2% sodium carbonate w/v) and covered the agar. The Petri dishes were sealed with Parafilm and then incubated at 28 °C for 48 hours. The observation was focused on a colour change in the filter paper, noting the transition from yellow to light brown, brown, or reddish-brown.

Conclusion

Plant Growth Promoting Rhizobacteria (PGPR) comprises a community of bacteria residing in the soil rhizospheric zone of plants. These bacteria exhibit the ability to directly or indirectly suppress plant diseases caused by various pathogens simultaneously promoting plant growth. The rhizobacterial isolates demonstrate both plant-growth-promoting and bio-control activities. These rhizobacteria exhibit significant potential for utilization as bio-pesticides and bio-fertilizers to enhance crop health and growth. Functioning as antagonistic agents these rhizobacteria play a crucial role in reducing disease incidence and contributing to overall crop production. As the demand for chemical residue-free agricultural products continues to rise there is a pressing need to explore and implement more effective and efficient bio-control agents in farming practices which can be effectively achieved by the use of PGPRs.

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