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Original Article

BURKHOLDERIA CEPACIA STRAIN IISRCLRB5, A PROMISING BIOAGENT FOR THE MANAGEMENT OF RHIZOME ROT OF TURMERIC (CURCUMA LONGA L.)

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ABSTRACT

The efficacies of 15 rhizobacterial isolates collected from different rhizosphere soils of ginger and turmeric were tested for their ability to inhibit the growth of three Pythium spp. viz., Pythium aphanidermatum, P. myriotylum and P. vexans causing rhizome rot of turmeric, ginger, and cardamom respectively. In vitro studies revealed that the isolate IISRCLRB5 was very inhibitory showing more than 70% inhibition when inoculated simultaneously with Pythium spp. and complete inhibition was obtained on sequential inoculation i.e., by inoculating bacteria first followed by inoculating Pythium spp. after 48 h on dual plate assay. Further in planta evaluation of the isolate against P. aphanidermatum in one month old turmeric plants revealed 56.67% reduction in disease incidence when the rhizobacterium and P. aphanidermatum inoculated simultaneously and 100% rhizome rot suppression when treated with rhizobacterium 48 h before the pathogen inoculation (sequential inoculation). The biocontrol activity of IISRCLRB5 was reconfirmed by another pot culture experiment using four months old turmeric plants and the activity was compared with the fungicide metalaxyl mancozeb-72% WP in the recommended concentration (0.125 g/ 100 mL). The isolate was subjected to various identification modules viz., phenotypic, biolog identification and molecular sequencing and identified as Burkholderia cepacia. The isolate was further studied for the plant growth promoting and pathogen-inhibiting properties. The isolate is found positive for nitrogen fixation, siderophore production, IAA production, ammonia production, phosphate solubilization, potassium utilization, α- amylase production and protease production.

KEYWORDS: *Bhurkolderia cepacia, Biocontrol Agent, Biolog, Curcuma longa L., IISRCLRB5, Inhibition, P. aphanidermatum, P. myriotylum, P. vexans, Phosphate Solubilization, Potassium Solubilization & Siderophore*

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1. INTRODUCTION

India, ''the land of spices'' is the largest producer, consumer, and exporter of spices in the world. Turmeric (*Curcuma longa* L.) "the golden spice of India", stands next to black pepper and cardamom in importance. In world trade, India ranks first in both production as well as export but its cultivation is severely afflicted by various diseases. Among the diseases, rhizome rot caused by *Pythium aphanidermatum*, an oomycete pathogen, is a major problem which leads to extensive yield loss in India (Ravindran et al., 2007). Studies by (Anoop and Suseela Bhai, 2009 & 2014) showed that rhizome rot of turmeric is primarily caused by *Pythium* species mainly *P. aphanidermatum* and all the other pathogens are secondary invaders. Even though chemicals show promising results in controlling the disease, development of fungicide resistance among the pathogens,

pesticide residues in food stuffs and the development of oncogenic risks have encouraged the exploitation of potential antagonistic microflora in disease management.

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial microbes inhabiting the rhizosphere of different crop plants (van Loon et al., 2008), displays antagonism (Beneduzi et al., 2012) as a direct mechanism and induced systemic resistance (ISR) as an indirect mechanism (Kloepper et al., 2004) in disease control. PGPR enhances plant growth through a wide range of mechanisms including various enzyme production which alters plant growth and development (Penrose and Glick, 2003), nutrients mobilization through biological nitrogen fixation (Bhattacharjee et al., 2008), various phytohormone secretion (Santner et al., 2009), enhances phosphorus uptake (Yazdani et al., 2009) and siderophore production (Rosenblueth and Martinez-Romero, 2006). Though several researchers reported the efficiency of biocontrol agents against various diseases, relatively very few researchers have attempted to study the effectiveness of the biocontrol agent against rhizome rot disease and its multiple functions related to turmeric production (Anoop and Suseela Bhai, 2014). Against this background, the objectives of this study were to isolate and characterize effective biocontrol agents based on *in vitro* and *in planta* antagonism to species of *Pythium* and to understand its biocontrol and growth promoting traits and hydrolytic enzymes.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples and Isolation of Bacteria

Rhizosphere soil samples were collected from healthy ginger and turmeric growing tracts of Kozhikode, Malappuram and Wayanad districts of Kerala and Nilgiris district of Tamil Nadu (India). The soil samples were subjected to serial dilution plating. The serial dilution was continued up to 10^{-10}, followed by pour plating on nutrient agar (NA) medium The plates were incubated at 28°C for 48-72 h. The discrete bacterial colonies were then selected and sub-cultured on to nutrient agar and preserved in 40 % glycerol for further studies.

2.2 *In vitro* Screening of Rhizobacteria for Antagonism against *Pythium* spp.

All the 15 rhizobacteria were screened using dual plate culture technique (Berg et al., 2005) against three species of *Pythium viz., P. aphanidermatum, P. myriotylum,* and *P. vexans,* the probable rhizome rot pathogens of turmeric, ginger and cardamom respectively. The *Pythium* cultures as mentioned above were isolated from respective crops and maintained in PDA were used for evaluation studies. The species of *Pythium* were sub cultured on to PDA and grown for 48 h. Mycelial plugs of 5 mm size cut from the periphery of this actively growing culture were used for *in vitro* inoculation. The test was done in three independent replications on Potato Dextrose Agar (PDA). For simultaneous inoculation studies, a mycelial plug of actively growing *Pythium* spp. was placed on to the center of the respective agar medium and the bacterial strain was streaked on either side of the mycelial plug. Plates were then incubated at 28°C for 72 h. For sequential inoculation studies, rhizobacterium inoculation was done 48h prior to pathogen inoculation. Plates inoculated with species of *Pythium* alone served as the control for both simultaneous and sequential studies. After incubation, the radial growth of *Pythium* spp. was measured and percent inhibition of growth over control was estimated using the formula, $I = [(C - T)/C] \times 100$, where I is the per cent inhibition and C and T are the radial growth of the pathogen in control and treatment, respectively. Rhizobacterial isolate that showed > 70 % inhibition of the pathogens was used for further studies.

2.3 *In Planta* Evaluation of Selected Rhizobacteria for Disease Suppression

For the *in planta* studies, disease free rhizomes of turmeric variety 'IISR Prathibha' were grown and maintained in sterile porting mixture (soil: sand: FYM, 2:2:1) in 12-inch pots. For rhizobacterial inoculum, 1 mL log phase bacterial culture of (3×10^{10} CFU mL^{-1}) was added to the nutrient broth and incubated at room temperature (25 ± 2°C) for 48 h. *P. aphanidermatum* grown for 72 h on PDA plates was macerated and 10 ml of the concentrate (1g mycelium) was then suspended in 100 ml of distilled water served as the pathogen inoculum. The isolate showed more than 95 % inhibition against all the three species of *Pythium in vitro* in sequential studies was used for the *in planta* study. Two pot culture experiments with Complete Randomized Design (CRD) were conducted to test the efficacy of IISRCLRB5 application against turmeric rhizome rot. Five treatments were included in the studies with three replications using the turmeric variety IISR-Prathibha (one month old plants) in the first experiment. The treatments were (1) absolute control (no bacteria, no pathogen), (2) IISRCLRB5 (plants treated with the bacterial suspension alone), (3) pathogen control (challenged with *P. aphanidermatum*), (4) IISRCLRB5 + pathogen (simultaneous inoculation) and (5) IISRCLRB5 + pathogen (pathogen inoculated 48hr after IISRCLRB5 treatment). For the second pot culture, experiment four months old turmeric plants in the rhizome development stage were used. The treatments include (1) absolute control, (2) pathogen control, (3) IISRCLRB5 + pathogen (pathogen inoculated 48 h after IISRCLRB5 treatment), and (4) metalaxyl mancozeb-72% WP (0.125 g/100 mL). Observations on rhizome rot incidence were recorded as root rot percentage in first pot culture experiment whereas in the second experiment the disease incidence was recorded. The percent incidence of root rot and rhizome rot was calculated by the formula,

Percent Root rot = (number of root infected/total number of roots) × 100

Percent Rhizome rot = (number of rhizomes infected/total number of rhizomes) × 100

2.4 Identification of Potential Bacterial Isolate

Based on the potential traits, the selected IISRCLRB5 was subjected to identification process using the Bergey's Manual of Determinative Bacteriology, Biolog Microstation System and 16S rDNA sequence analysis. The selected isolate was tentatively identified by phenotypic identification using the Bergey's Manual of Determinative Bacteriology such as Gram staining, motility, indole production test, methyl red test, VP test, citrate utilization test, the presence of oxidase, urease and catalase, casein hydrolysis, starch hydrolysis, gelatin liquefaction, ammonia production, HCN production, H_2S production on triple sugar iron agar, glucose fermentation and growth at 1%, 2%, 5%, and 10% salt concentration were assessed for each rhizobacteria as described by (Holt et al., 1994; Tindal et al., 2007). The identity IISRCLRB5 was confirmed using Biolog Microstation System (RDG Laboratories, Hayward, California, USA). The identity was further confirmed using 16S rDNA sequence analysis. For that, the genomic DNA from the isolate was extracted using the standard protocol (Sambrook and David, 2000). The 16S rDNA gene was amplified using a universal primer set 1492R 5' TACGGYTACCTTGTTACGACTT 3' and 27F 5' AGAGTTTGATCMTGGCTCAG 3'. The final PCR product was resolved in 1 % agarose gel, excised and purified with elution kit (Sigma, India). The DNA sequencing was performed with Scigenom Labs, Cochin (India) and subjected to BLAST analysis. Partial sequence data for the 16S rDNA gene was deposited in GenBank (NCBI) nucleotide sequence data-base library.

2.5 Characterization of Short Listed Rhizobacteria for Beneficial Traits

Jenson's medium, the selective medium for the growth of nitrogen-fixing bacteria (Jensen, 1954) was used to assess the trait of nitrogen fixation. The IISRCLRB5 was streaked on to Jensen's media plates and observed for growth at 48 h. The growth of bacterium on the Jenson's agar plate indicates a positive result. Siderophore production was observed by the method of Jalal and van Helm, 1991. The appearance of orange or reddish brown color indicated the presence of siderophores. The isolate was tested for its ability to produce IAA by the method of (Sarwar and Kremer, 1995). Formation of red color in the suspension indicated the production of IAA by the organism. Cappuccino and Sherman (1992) method was followed for NH_3 production. The formation of faint yellow to deep yellow or to the dark brown color indicated the production of NH_3. HCN production was measured by the qualitative method of (Kloepper et al., 1991) with slight modifications. 25 µL of log phase cultures of the bacteria were inoculated into 5 mL of King's B broth supplemented with 4.4 gL^{-1} of glycine taken in 30 mL sterile glass vials. Picric acid solution (2.5 g picric acid + 12.5 g Na_2CO_3 in 1 L distilled H_2O) impregnated filter paper strip was inserted in half of the vials and tightened with a screw cap. The parafilm sealed vial was incubated for 72 h in a mechanical shaker. The change in color of the filter paper strips from yellow to brown to red indicates the production of HCN. No change in color of the filter paper strip indicates a negative test.

A loop full of the 24 h broth culture was spot inoculated on the center of Pikovskayas agar plates and were incubated at 28°C for 96 h (Gaur, 1990) for phosphate solubilization studies. The zone of clearance around the bacterial colony on incubation indicated the solubilization of phosphate. Potassium solubilization was assessed using modified Aleksandrov medium (Rajawat et al., 2016). The plates with a bright yellow zone of clearance around the wells indicated the solubilization of potassium by the organism. Zn solubilization was determined by the method described by Saravanan et al., (2004) in mineral salts agar medium and observed for solubilization 15 days after inoculation. An absence of solubilization zone indicated a negative result. Cell-wall-degrading enzyme production (α-amylase, protease, pectinase, and cellulase) was observed by the methods described by Cappuccino and Sherman, (1992). For determining the production of α-amylase one loopful of the bacterial cell suspension was streaked on the starch agar plate and incubated in an inverted position for 48 hrs at 37°C. Lugol's iodine solution was poured on to the incubated starch agar plate and observed for the clear zone. For the determination of protease, the isolate was spot inoculated on to protease skim milk agar plate. After 48 h of incubation at 28°C, the plates were observed for clear zone around the bacterial growth. For the determination of pectinase and cellulase by the isolate, the media was prepared by adding 1 % pectin and 1 % cellulase in basal medium ($NaNO_3$ – 1 g, K_2HPO_4 – 1 g, KCl – 1 g, $MgSO_4$ – 0.5 g, yeast extract – 0.5 g, glucose – 1 g, distilled water – 1000 mL, Agar – 15 g) separately. One loop full of the bacterial cell suspension was spot inoculated on to the center of each medium and incubated for 5 days at 37°C. Gram's iodine solution was poured in the pectin agar and observed for the zone of clearance against the dark blue background for pectinase production. The cellulose medium was flooded with 0.01% Congo red solution for 15 min and the plates were de-stained using 1 % NaCl solution for 5 min and observed for the zone of clearance of cellulase. An absence of zone of clearance marks negative test in both.

2.6 Statistical Analysis

The significance of treatment effects wherever applicable was determined by one-way analysis of variance (ANOVA). Comparisons of means were made using the Least Significance Test (LSD) at 0.05 probability level by using SSCNARS of IASRI. All the experiments were repeated twice for confirmation.

3. RESULTS

A total of 15 rhizobacteria were isolated from ten soil samples collected from healthy tracts of ginger and turmeric where seven isolates were from ginger rhizosphere and eight isolates from turmeric rhizosphere from Kerala and one isolate each from turmeric and ginger from Tamil Nadu (India). The isolates were nomenclatured as IISRGRB for ginger rhizosphere bacteria and IISRCLRB for turmeric rhizosphere bacteria (G stands for ginger, RB stands for rhizosphere bacteria, CL stands for *Curcuma longa* and IISR represents the ICAR-Indian Institute of Spices Research where the study is undertaken). The details of the isolates are given in Table 1.

Table 1: Details of Rhizobacteria Isolated from Various Locations of South India

Sl. No.	Crop	Place of collection	Latitude/longitude	Isolate code
1	Ginger	Chelavoor, Kozhikode	11°17'51.576"N/75°51'3.5388"E	IISRGRB1
2	Turmeric	Chelavoor, Kozhikode	11°17'51.576"N/75°51'3.5388"E	IISRCLRB2
3	Ginger	Olavanna, Kozhikode	11°14'3.12"N/ 75°49'5.0484"E	IISRGRB3
4	Ginger	Olavanna, Kozhikode	11°14'3.12"N/ 75°49'5.0484"E	IISRGRB4
5	Turmeric	Olavanna, Kozhikode	11°14'3.12"N/ 75°49'5.0484"E	IISRCLRB5
6	Ginger	Sultanbathery, Wayanad	11°43'32.20"N/ 76°6'39.49"E	IISRGRB6
7	Turmeric	Sultanbathery, Wayanad	11°43'32.20"N/ 76°6'39.49"E	IISRCLRB7
8	Ginger	Gudallur, Nilgiris	11°30'2.56"N/ 76°29'31.52"E	IISRGRB8
9	Turmeric	Gudallur, Nilgiris	11°30'2.56"N/ 76°29'31.52"E	IISRCLRB9
10	Ginger	Cheekode, Malappuram	11°14'3.12"N/ 75°49'5.0484"E	IISRGRB10
11	Ginger	Cheekode, Malappuram	11°14'3.12"N/ 75°49'5.0484"E	IISR GRB11
12	Turmeric	Cheekode, Malappuram	11°14'3.12"N/ 75°49'5.0484"E	IISRCLRB12
13	Turmeric	Cheekode, Malappuram	11°14'3.12"N/ 75°49'5.0484"E	IISRCLRB13
14	Turmeric	Mananthavady, Wayanad	11°48'8.60"N/ 76°0'13.98"E	IISRCLRB14
15	Turmeric	Mananthavady, Wayanad	11°48'8.60"N/ 76°0'13.98"E	IISRCLRB15

3.1 *In vitro* Screening of Rhizobacteria for Antagonism against *Pythium* spp.

To get a promising biocontrol agent, all the 15 rhizobacteria were screened for inhibition *in vitro* against *Pythium* spp. *viz.*, *P. aphanidermatum*, *P. myriotylum* and *P. vexans* by dual culture method. Among the isolates, IISRCLRB5 showed more than 70 % inhibition over control (71.81 % against *P. aphanidermatum*, 74.07 % against *P. myriotylum* and 81.48 % against *P. vexans*) when inoculated simultaneously. A complete (100 %) inhibition was observed against all the three tested species of *Pythium* spp. on sequential inoculation where bacteria were inoculated 48 h prior to fungal inoculation. No significant inhibition was observed with any other isolates tested against any of the three *Pythium* species. The details of *in vitro* screening are given in Table 2. The results clearly showed the antagonistic potential of IISRCLRB5 and hence this isolate was selected for further studies. The observed antagonistic activity of IISRCLRB5 against all the three pathogens is shown in Figure 1.

Table 2: *In vitro* Screening of Rhizobacteria against *Pythium* spp

Sl. No.	Isolate Code	% Inhibition					
		P. aphanidermatum		*P. myriotylum*		*P. vexans*	
		Simultaneous	Sequential	Simultaneous	Sequential	Simultaneous	Sequential
1	IISRGRB1	5.18 (13.09)	6.66 (14.82)	5.92 (13.89)	5.92 (14.03)	5.18 (13.09)	5.89 (14.03)
2	IISRCLRB2	11.85 (20.12)	11.85 (20.12)	11.85 (20.01)	12.59 (20.76)	11.85 (20.12)	13.33 (21.41)
3	IISRGRB3	18.51 (25.48)	19.26 (26.02)	14.81 (22.58)	16.29 (23.79)	16.29 (23.76)	18.51 (25.48)

Table 2: Contd.,							
4	IISRGRB4	0 (1.65)	0 (1.65)	2.96 (12.16)	4.44 (12.16)	0 (1.65)	5.92 (14.03)
5	IISRCLRB5	74.81 (59.88)	100 (88.35)	74.07 (59.40)	100 (88.35)	81.47 (64.57)	100 (88.35)
6	IISRGRB6	17.77 (24.93)	18.51 (25.48)	15.55 (23.19)	19.26 (26.02)	17.77 (24.91)	20 (26.57)
7	IISRCLRB7	19.26 (18.76)	11.85 (20.12)	12.59 (20.76)	13.33 (21.41)	14.07 (22.01)	15.55 (23.22)
8	IISRGRB8	19.26 (26.02)	19.26 (26.02)	12.59 (25.48)	20 (26.57)	16.29 (23.79)	16.29 (25.48)
9	IISRCLRB9	21.48 (27.60)	21.48 (27.60)	14.81 (22.35)	18.51 (25.42)	14.07 (22.01)	16.29 (23.79)
10	IISRGRB10	14.07 (22.01)	14.81 (22.62)	14.81 (22.62)	16.29 (23.79)	17.03 (24.36)	18.51 (25.48)
11	IISRGRB11	0 (1.65)	0 (1.65)	0 (1.65)	5.92 (13.09)	5.18 (13.09)	8.14 (16.46)
12	IISRCLRB12	5.92 (14.03)	9.62 (18.05)	8.88 (17.26)	10.37 (18.76)	9.62 (17.97)	12.59 (20.76)
13	IISRCLRB13	0 (1.65)	0 (1.65)	0 (1.65)	5. 18 (13.09)	0 (1.65)	5.92 (14.03)
14	IISRCLRB14	17.03 (24.36)	16.29 (23.79)	15.55 (23.22)	18.51 (25.48)	17.03 (24.36)	19.26 (26.02)
15	IISRCLRB15	13.33 (21.41)	17.03 (24.36)	14.81 (22.62)	18.51 (25.48)	16.29 (23.79)	19.26 (26.02)
	LSD at 5%	1.574	1.7896	3.3381	1.9932	2.4532	1.9886

*Values given in parenthesis are arcsine transformed values

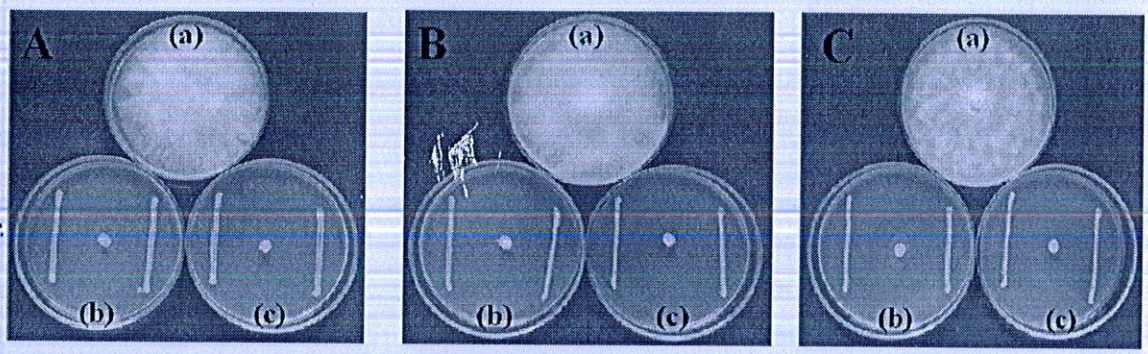

Figure 1: Antagonistic Activity of IISRCLRB5 against (A) *P. aphanidermatum*, (B) *P. myriotylum* and (C) *P. vexans;* (a) Control plate (pathogen alone), (b) Simultaneous inoculation of bacterium and pathogen, (c) Sequential inoculation (bacterium inoculated 2 days before the pathogen)

3.2 *In Planta* Evaluation of Promising PGPR for Disease Suppression

The selected isolate was evaluated for their disease suppression activity on turmeric under greenhouse conditions in two pot culture experiments. The plants showed disease symptoms from the 5th day of inoculation in the first experiment using one-month-old turmeric plants. Observation on root rot percentage was recorded after twelve days of inoculation and statistically analyzed. Under greenhouse conditions, the plants treated with IISRCLRB5 two days before the pathogen inoculation (sequential inoculation) recorded 100 % rhizome rot control. But only 56.67 % suppression was recorded in plants treated simultaneously with IISRCLRB5 and pathogen. Negative control showed 100 % disease incidence on twelfth day (Table 3 and Figure 2). Both absolute control and plants treated with bacteria alone remained healthy with no rhizome rot incidence. In the second pot culture experiment, plants showed disease symptoms from the 8th day of inoculation. Observation on root rot percentage and rhizome rot percentage were recorded after 30 days of inoculation and statistically

analyzed. Under greenhouse conditions, the plants treated with IISRCLRB5 two days before the pathogen recorded 100 % rhizome rot control. Negative control showed 100 % rhizome rot incidence (Table 4 and Figure 3). In absolute control, plants remained healthy with no rhizome rot incidence. The *in planta* test clearly revealed the potential of IISRCLRB5 in suppressing the predominant rhizome rot pathogen on turmeric *viz., P. aphanidermatum.* The plants treated with metalaxyl mancozeb in recommended concentration showed 19.39 root rot incidence and 6.67 % rhizome rot incidence. So the *in planta* tests clearly revealed the potential of IISRCLRB5 in suppressing the predominant rhizome rot pathogen on turmeric *viz., P. aphanidermatum.*

Table 3: Effect of IISRCLRB5 on Rhizome Rot Incidence of Turmeric in First pot experiment

Sl. No.	Treatment	Root Rot (in Percent)
1	Absolute control	0 (1.65)
2	Positive control	0 (1.65)
3	Negative control	100 (88.35)
4	Simultaneous inoculation	56.67 (48.85)
5	Sequential inoculation	0 (1.65)
	LSD at 5 %	3.0695

*Values given in parenthesis are arcsine transformed values

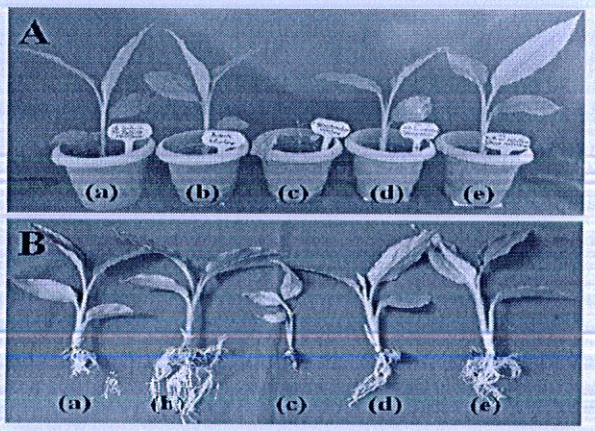

Figure 2: Effect of IISRCLRB5 on *Pythium* Infection in Turmeric (First pot experiment), (A) in pot, (B) up rooted; (a)Absolute control, (b) Positive control, (c) Negative control (*Pythium*), (d) Simultaneous inoculation, (e) Sequential inoculation

Table 4: Effect of IISRCLRB5 on Rhizome rot of Turmeric in Second pot experiment

Sl. No.	Treatment	Root rot (in percent)	Rhizome rot (in percentage)
1	Absolute control	0 (1.65)	0 (1.65)
2	Pathogen control	100 (88.35)	100 (88.35)
3	IISRCLRB5 + pathogen	0 (1.65)	0 (1.65)
4	Metalaxyl mancozeb 72 % (0.125 g/100 mL)	19.39 (25.70)	6.67 (9.96)
	LSD at 5 %	6.3292	13.545

*Values given in parenthesis are arcsine transformed values

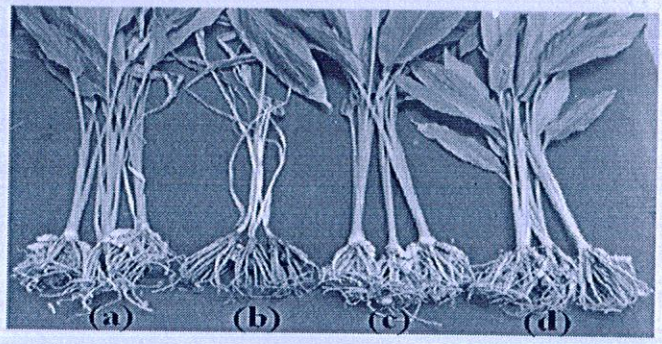

Figure 3: Effect of IISRCLRB5 on *Pythium* Infection in Turmeric (Second pot experiment), (a) Absolute Control, (b) Pathogen Control, (c) IISRCLRB5+ Pathogen, and (d) Metalaxyl Mancozeb-72 % (0.125 g/100 mL)

3.3 Identification of IISRCLRB5

Based on phenotypic and biochemical characters the isolate was identified as *Burkholderia* spp. as per Bergey's manual of Determinative Bacteriology. The details are given in Table 3.

Table 5: Details of Phenotypic and Biochemical Tests *in vitro*

Traits	Activity	Traits	Activity
Gram's reaction	Gram negative	Starch hydrolysis	+
Motility	Motile	Gelatin liquefaction	-
Indole production	-	Ammonia production	+
Methyl red	+	HCN production	-
Voges- Proskauer	-	H_2S production on TSI	+
Citrate utilization	+	Glucose fermentation	+
Catalase test	+	1% NaCl	+
Urease test	+	2 % NaCl	+
Oxidase test	+	5 % NaCl	-
Casein hydrolysis	+	10 % NaCl	-

By the Biolog based identification using all the three protocols using IFA, IFB and IFC –GEN III inoculation fluids, the isolate was identified as *Burkholderia cepacia*. The identity was further confirmed by 16SrDNA sequence analysis using the primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'). The partial sequence data for the 16S rDNA gene (Table 6) was deposited in GenBank (NCBI) nucleotide sequence data base library. The sequence was deposited in NCBI with accession number MK056183.

Table 6: The Partial Sequence of the 16SrDNA Gene of *B. cepacia* MK056183

CAGTCGACGGCAGCACGGGTGCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGTAATACATCGGAACAT
GTCCTGTAGTGGGGGATAGCCCGGCGAAAGCCGGATTAATACCGCATACGATCTACGGATGAAAGCGGGG
GACCTTCGGGCCTCGCGCTATAGGGTTGGCCGATGGCTGATTAGCTAGTTGGTGGGGTAAAGGCCTACCA
AGGCGACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAG
AAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATCCTTGACTCTAATACAGTCGGGGGATGAC
GGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTA
ATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTGCTAAGACCGATGTGAAATCCCCGGGCTCAAC

CTGGGAACTGCATTGGTGACTGGCAGGCTAGAGTATGGCAGAGGGGGGTAGAATTCCACGTGTAGCAGTG
AAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGCCAATACTGACGCTCATGCA
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTG

3.4 Screening IISRCLRB5 for Beneficial Traits and Hydrolytic Enzymes

The details of *in vitro* screening for beneficial traits and hydrolytic enzymes are given in Table 7 and Figure 4.

Table 7: Details of Production of Beneficial Traits and Hydrolytic Enzymes *in vitro*

Traits	Activity	Traits	Activity
Nitrogen fixation	+	α- amylase production	+
Siderophore production	+	Protease production	+
IAA production	+	Zinc solubilization	-
Phosphate solubilization	+	Cellulase production	-
Potassium utilization	+	Pectinase production	-

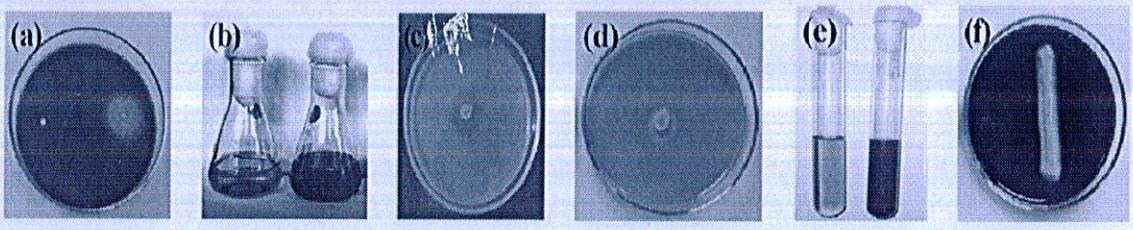

Figure 4: *In vitro* results of (a) Potassium solubilization, (b) Ammonia production, (c) Phosphate solubilization, (d) Caseinase production, (e)Siderophore production, (f) Amylase production

4. DISCUSSIONS AND CONCLUSIONS

Rhizome rot of turmeric remains as a serious malady in many of the turmeric growing tracts where chemical fungicides having residual effects are being used to control the disease. The residual effects of the plant protection chemicals may hamper the quality of curcumin or other essential oils which imparts the properties of turmeric. Because of its eco-friendly nature and safety, the use of microbial biocontrol agents is an alternative approach for reducing the use of chemicals in agriculture and they might interact more closely with the host plants and act as effective biocontrol agents in sustainable crop production. So the present study focused on isolating a potential environmentally safe bioagent for combating rhizome rot based on *in vitro* and *in planta* antagonism to species of *Pythium* and to understand its beneficial effects. In order to select a potential antagonist against *Pythium* spp. a multistep screening for biocontrol activity, growth promoting traits and production of hydrolytic enzymes was done. Based on this one rhizobacterium was isolated and characterized as a potential candidate and identified as *B. cepacia.*

B. cepacia has been reported to prevent *Pythium* diseases of cucumber and peas and *R. solani* stem rot of poinsettia (Cartwright and Benson, 1995). The organism *B. cepacia* has shown effective in suppressing many plant diseases and also promote plant growth (Govindarajan et al., 2008). Scuderi et al., 2009 reported that *B. gladioli* and *B. cepacia* showed *in vitro* and *in vivo* antagonistic activity against blue and green moulds of citrus and apple fruits. de Los Santos et al., 2012 reported *Burkholderia cepacia* XXVI as a promising biological control agent against *C. gloeosporioides,* the causal agent of anthracnose through the production of hydroxamate siderophore. Previous studies on PGPR demonstrated that a single potential strain exhibits multiple traits related to biocontrol activity and growth

promotion both *in vitro* and *in vivo* (Praveen Kumar et al., 2014). Production of cell wall degrading enzymes (glucanase, chitinase, protease) and production of secondary metabolites (Siderophore and HCN) are common mechanisms that bacteria use to inhibit fungal pathogens (Indira et al., 2011).

Based on the assessment scheme, *B. cepacia* strain IISRCLRB5 is found to be nitrogen fixing, phosphate solubilizing and potassium utilizing. It also produced hydrolytic enzymes and plant growth promoting traits. Also, the bacterium is showing a positive correlation in *in vitro* and *in vivo* studies against *P. aphanidermatum*. Our findings confirm that *Burkholderia cepacia* strain IISRCLRB5 could be considered as a promising biocontrol candidate which can control rhizome rot diseases of turmeric. In further experiments, these selected BCA would be evaluated in a field trial to reconfirm the activity and propose for commercialization.

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