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Characterization of UV-B Tolerant Plant Growth Promoting Rhizobacteria (PGPR)and their effect on growth characteristics of Curcuma longa

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ABSTRACT
Six bacterial isolates were isolated from rhizospheric zone of Curcuma longa L. were characterized on the basis of morphology and biochemical characteristics. The isolates were efficient in producing PGP compounds like IAA, Ammmonia, HCN, Catalase and Siderophore and designated as PGB1, PGB3, PGB5, PGB7, PGB9 and PGB10. Prior to mother rhizome grown in pots, mother rhizomes were treated with PGPR isolates and plants were harvested after 21 days of inoculation. Results of first study showed that PGPR inoculation significantly enhanced rhizome germination and rhizome vigour of Curcuma longa. Also application of PGPR isolates significantly improved the percentage of rhizome germination and vigour index under UV-B exposure (60 minutes). Subsequently to investigate the effect of PGPR isolates on the growth characteristics, a pot culture experiment was conducted and plants were harvested after 60 days of inoculation. Most of the isolates resulted in a significant increase of plant height, fresh and dry weight, and leaf area and leaf number. Results confirmed that bacterial inoculation had significant effect on stimulation of root and shoot growth. Our findings suggest that the use of PGPR isolates PGB10, PGB9 and PGB7 as inoculants biofertilizers might be beneficial for growth of Curcuma longa even under UV-B stress.

Keywords: IAA, Ammonia, HCN, PGPR and Curcuma longa.

I. INTRODUCTION

Curcuma longa L. commonly known as turmeric is a rhizotomous herb of family Zingiberaceae cultivated in Indian sub-continent and the Middle East countries. The mature dried rhizome is most common ingredient of Indian kitchen as spice and well known antiseptic, antipyretic since ancient times. The medicinal properties are assigned due to the presence of curcuminiod and sesquitepenoid compounds. Curcumin, the most important curcuminoid, is used as antioxidant, antimicrobial, anti-inflammatory and is even effective against cancer and HIV.

A great deal of interest has been generated on studies related to increments in UV-B radiation in recent years. A decrease in the concentration of stratospheric ozone is enhancing the solar ultraviolet-B radiation on the earth's surface [1]. Bacteria are particularly vulnerable to UV-B damage because of their small size limits, effective cellular shading or protective pigmentation and their genetic material comprises a significant portion of their cellular volume [2]. Results from field studies on rhizobacteria indicates that exposure to natural solar UV-B radiation results in a decrease in total cell abundance, a reduction in amino acids uptake, a depression of the activity of degrading enzymes and a significant inhibition of protein and DNA synthesis. Several studies have indicated that UV-B radiation can deleteriously affect the soil microbial diversity, physiological processes and overall growth in a number of plant species [3, 4]. Romanovskaia, [5] reported that total number of bacteria and the number of dominant species in soil samples exposed to UV-B radiation decreased. Thus, indicating the unfavourable effect of UV-B radiations on rhizobacteria and their plant growth promoting (PGPR) activities. The number of rhizobacteria bacteria in the rhizosphere soil and rhizoplane increases due to the progressive interaction between the roots and the microorganisms accompanied by continuous availability of nutrients for the growth of the microorganisms [6]. Plant growth promoting

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rhizobacteria are heterogenous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly or indirectly. The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on plant [7].

Curcuma longa L. is an important plant from medicinal point of view and UV-B has unfavorable effect on plant growth and its rhizobial associate. Thus, the present study was aimed to assess the effect of UV-B tolerant plant growth promoting rhizobacteria (PGPR) on seed germination and growth of Curcuma longa.

II. MATERIALS AND METHODS

Sampling and Isolation

The rhizospheric soil samples were collected from UV-B treated pots growing Curcuma longa from west of Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India. Randomly selected plants were uprooted carefully and the excess of soil was removed by gentle shaking and the soil adhering to roots formed composite samples. The collected samples were placed in plastic bags and kept at 40 ^{0}C in the laboratory until processed. 10 gram of UV-B treated rhizospheric soil of Curcuma longa were taken into 250 ml of conical flask and 90 ml of sterile distilled water was added to it. After serial dilution up to 10-7 an aliquot of their suspension was spread on the plates of Luria Bartany (LB) agar medium. After 3 days of incubation at 28 ^{0}C bacterial colonies were spread to other LB agar plates and incubated at 28^0 C for 3 days.

Morphological and Biochemical Characterization

Well isolated colonies were picked up and different characteristics of colonies such as shape, size, elevation, surface margin, colour, colony form, texture, pigmentation, odour and opacity etc. [9] were recorded. Selected isolates were biochemically characterized by Gram's reaction, carbohydrate fermentation, oxidase test, O-F test, H2S production, IMVIC tests, NO2 reduction and starch and gelatin hydrolysis as per the standard methods [10]. Motility of bacteria was observed by Hanging Drop method. A loopful of 2-day old culture was suspended in 1ml of nigrosin solution. A drop of suspension was taken on a cover slip. The cover slip was hanged on a hollow slide with Vaseline. The slide was then observed under microscope to test the motility of bacteria.

Characterization of Rhizobacteria for PGP Traits

Selected rhizobacterial isolates were characterized for plant growth promoting characteristics based on the standard procedures; IAA production was estimated by method of Bric [11] Ammonia, Catalase and HCN production by method of Bakker 1987 [12] and Siderophore production by method of Schwyn and Neilands [13]. Six potential isolates were selected on the basis of multiple PGP traits and were designated as PGB1, PGB3, PGB5, PGB7, PGB9 and PGB10. The culture of six isolates were streaked on LB agar plates and incubated at 10, 20, 28, 37 and 45^0 C.

Sowing

Mother rhizomes of Swarna variety were treated with mancozeb 75%, trade name dithane-M-45 @ 2.5 g per liter of water and endosulfan 2.0 ml per liter of water for minutes and were used for planting. Rhizomes were planted 7.5 cm down the pot ridge. After 21 days the number of germinated rhizomes was counted. Root and shoot length of individual rhizome was measured to determine the vigor index with following formula: Vigor index= (mean root length +mean shoot length) × % germination [14]. For evaluation of growth promotion with PGPRs, above bacterial strains were tested in soil conditions. The pots with 14 inch diameter and capacity to hold 8 Kg of soil were taken and PGPR inoculated rhizomes were sown at 7.5cm depth of soil in each pot. Treatments were arranged in a factorial experiment based on completely randomized design. All pots were watered uniformly, and no artificial fertilizers were used. After 60 days, fresh weight was determined and dry weight calculated by drying plants in an oven at 75°C until the weight remained constant. For leaf area determination, the area of each expanded leaf was calculated as K×length×width, where k =0.75 [15].

III. RESULTS AND DISCUSSION

Microbial diversity in soil is considered important for maintaining the sustainability of agriculture production systems [7].The quantity and activity of microorganisms are determining factor of the productivity of any kind of soil [16]. Near about 75 rhizobacterial isolates were isolated from the rhizosphere of Curcuma longa. After screening 6 potential isolates were selected for the present study and were designated as PGB1, PGB3, PGB5, PGB7, PGB9 and PGB10. As shown in Table 1, the morphological and biochemical characteristics of PGPR isolates varied widely. All the isolates were rod shaped with raised colonies having shiny surface and smooth margins. They differ in colour but all were odourless and no pigmentation was

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observed in the colonies of LB agar plates. Diameters of the colonies isolated varied from 0.2-2.0 mm. All the isolates were gram positive and most of them showed positive results for catalase, oxidase, O-F test, and starch hydrolysis and nitrate reductase. Results obtained in the present investigation were in agreement with the previous studies [17, 18, and 19]. Growth of isolates on LB agar plates varied with the temperature (Table 2). The growth of all isolates was good in the temperature range of 20 ^{0}C to 28 ^{0}C. In addition, PGB3 showed maximum tolerance to temperature (45 ^{0}C). Plant rhizosphere is known to be preferred ecological niche for soil microorganisms due to nutrient rich availability. Plant growth promoting activities such as IAA production, ammonia, catalase, siderophore etc. are the characteristics of plant growth promoting rhizobacteria (PGPR). In the present study also IAA, Ammonia, Catalase and HCN production was shown by all (100%) the isolates. Among them PGB7, PGB9 and PGB10 were strong IAA, Ammonia, Catalase and HCN producers, thus indicating their potential for plant growth promoting effects. However, production of Siderophore was detected less frequently than other PGP characteristics (Table 3). Among all the six isolates, PGB9 and PGB10 were strong siderophores producers. IAA secreted by a bacterium may promote root growth directly by stimulating plant cell elongation or division. Production of IAA by rhizobacterial isolates is also detected by other workers in Bacillus, Pseudomonas and other rhizobacterial isolates [18, 20, and 21]. Another important trait of PGPR, that may indirectly influence the plant growth, is the production of ammonia. Siderophore chelates iron and other metals contribute to disease suppression by conferring a competitive advantage to bio control agents for the limited supply of essential trace minerals in natural habitats [22, 23]. Production of catalase was exhibited by all the isolates of rhizobacteria. Catalase activity was detected in all the bacterial strains that may be potentially very advantageous bacterial strains showing catalase activity must be highly resistant to environmental, mechanical, and chemical stress. However, Phosphate Solubilization activity was not detected in any of the rhizobacterial isolates under study (data not shown).

Table.1: Morphological and Cultural Characteristics of PGPR Isolates

	PGP ISOLATES					
	PGB1	**PGB3**	**PGB5**	**PGB7**	**PGB9**	**PG10**
Gram's Reaction	+ve	+ve	+ve	+ve	+ve	+ve
Cell Shape	Rod	Rod	Rod	Rod	Rod	Rod
Size (mm)	0.9-1.1	1.0-1.6	1.7-2.1	1.6-2.0	0.8-1.0	0.6-1.1
Elevation	Raised	Raised	Raised	Raised	Raised	Raised
Colour	Off-white	Brown	Brown	Yellow	Yellow	Off-white
Pigmentation	None	None	None	None	None	None
Oxidase	+	+	-	+	+	+
OF- test	+	+	-	+	+	+
H2S Production	+	+	-	-	+	+
Indole	-	-	-	-	+	+
Methyl Red	-	-	-	+	+	+
Citrate Utilization	+	+	+	+	+	+
Nitrate Reduction	+	+	+	+	+	+
Starch Hydrolysis	-	-	+	+	+	+
Gelatin Hydrolysis	-	+	-	-	-	-
Vogues Proskauer	+	-	+	+	+	+

Table.2: Growth of PGPR Isolates at Different Temperatures

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	Temperature				
PGP Isolates	10^0 C	20 ^{0} C	28 ^{0} C	37 ^{0} C	45 ^{0} C
PGB1	+	++	+++	+	-
PGB3	+	++	+++	++	+
PGB5	+	++	+++	+	-
PGB7	-	++	+++	-	-
PGB9	+	++	+++	+	-
PGB10	-	++	+++	++	-

Table 3: Plant Growth Promoting Characteristics of Rhizobial Isolates

PGP Isolates	**PGP Characteristics**				
	IAA	**HCN**	**SIDEROPHORE**	**AMMONIA**	**CATALASE**
PGB1	++	+	-	+	+
PGB3	++	++	-	++	+
PGB5	++	++	-	++	+
PGB7	+++	++	+	++	+
PGB9	++++	+++	++	+++	++
PGB10	++++	+++	+++	++++	+

Some of the above-tested isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. Similar to our findings of multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored [19, 24]. Plant growth promoting effects of PGPR strains in different crops were clearly demonstrated [25]. Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease [26]. In the present study also UV-B exposure significantly decreased rhizome germination and vigor index in Curcuma longa. Effect of PGPR on germination percentage and vigor index also varied with bacterial isolates (Fig. 1 and Fig. 2). The effect of PGPR on germination rate of Curcuma longa plants under UV-B exposure was statistically significant at $p<0.05$.This present investigation confirms the earlier works. Previous studies have documented adverse effects of UV-B on plant growth. Similar results were obtained by Sharon [27] while working on gamma irradiation on seedling growth of Punica granatum. Under in vitro conditions seed treatment with PGPR strains improved seed germination; seedling vigor, seedling emergence and seedling stand over the control both with UV-B and without UV-B. Similar results were found in in earlier reports [28, 29, 30, 31, and 32].

Fig.1 Effect of PGPR inoculation on Germination Rate of Curcuma longa under UV-B exposure (60 minutes) after 21 days of inoculation.

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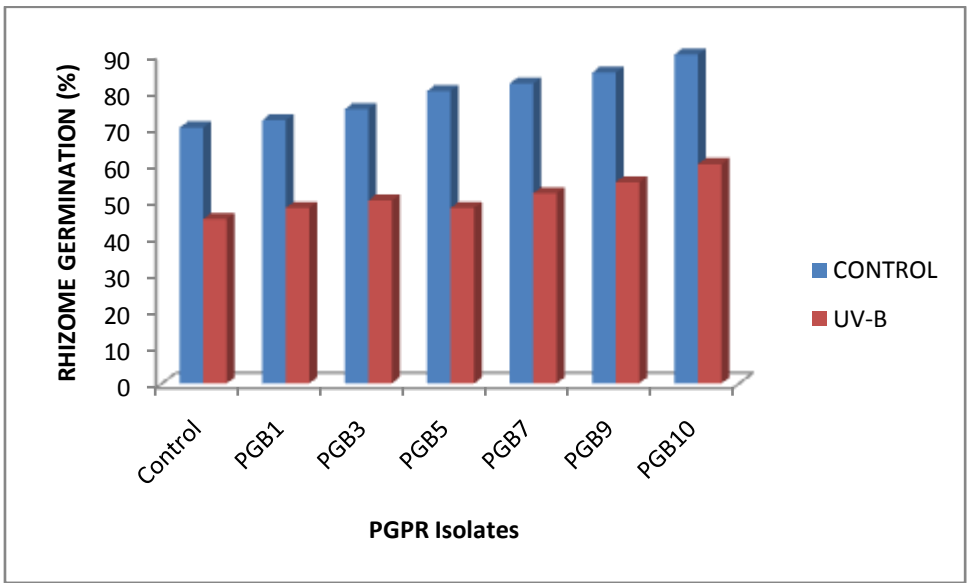
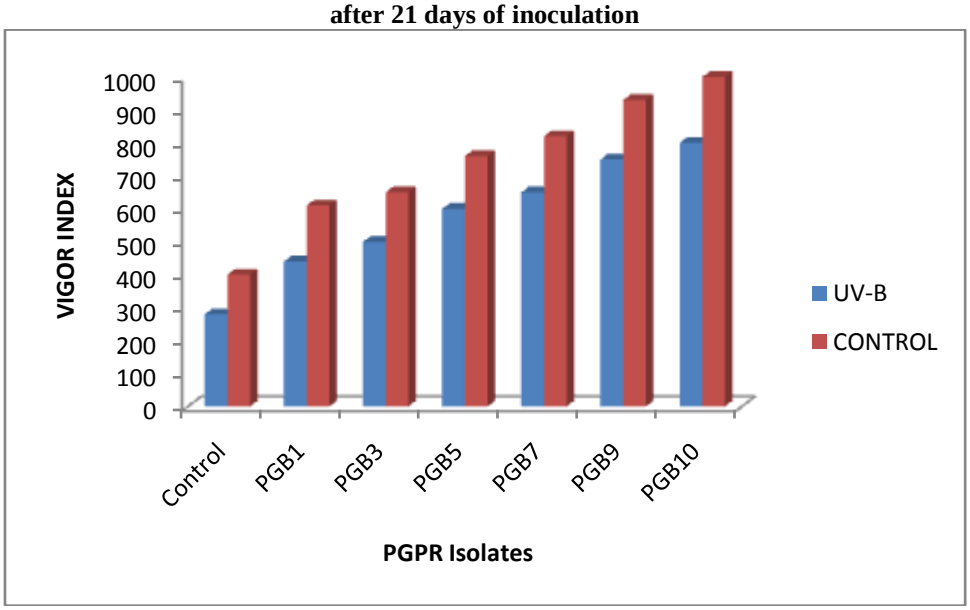


Fig. 2 Effect of PGPR inoculation on Vigor Index of Curcuma longa under UV-B exposure (60 minutes) after 21 days of inoculation



In pot experiment also, PGPR strains significantly enhanced plant growth as compare to control. Burd et al. [33] reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens. Our results also revealed that plant height increased by 37%, 35% and 33% by isolates PGB10, PGB9 and PGB7 respectively. Khalid et al., [34] showed that responses of wheat growth to inoculation with rhizobacteria depend on plant genotype and PGPR strains as well as environmental conditions. Observed data are presented in Table 4. Fresh weight and dry weight of Curcuma longa was significantly increased up to 17% and 14% by PGB10 and PGB9 respectively. PGB10 produced highest plant biomass i.e. fresh and dry weight followed by PGB9, PGB7, PGB5 and PGB3. Most of the isolates significantly increased other growth characteristics such as leaf number, leaf area etc.

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Table 4: Effect of PGPR Inoculation on Growth Characteristics of Curcuma longa plants at 60 days after sowing

TREATMENTS	Plant height (cm)	No. of leaves	Leaf Area (dm^2)	Fresh Weight (g/plant)	Dry Weight (g/plant)
Control	28.07^{f}	8^{f}	23.69^{e}	93.40^{g}	38.45^{g}
PGB1	29.98^{e}	8.5^{ef}	24.65^{d}	95.70^{f}	40.08^{f}
PGB3	31.09^{d}	9^{e}	25.01^{c}	$102.\ 34^{e}$	40.83^{e}
PGB5	31.66^{d}	9^{a}	25.46^{bc}	103.65^{d}	41.45^{d}
PGB7	32.78^{c}	9.5^{d}	25.63^{bc}	108.55^{b}	46.75^{c}
PGB9	34.46^{b}	10^{c}	25.84^{ab}	110.66^{b}	47.57^{b}
PGB10	35.56^{a}	10.5^{b}	26.13^{a}	112.56^{a}	48.22^{a}
SE±	0.181	0.116	0.047	0.020	0.008
CD	1.008	0.805	0.0498	0.375	0.146

Data are means ± standard error of three independent experiments. Different letters show significant difference at $P<0.05$

IV. CONCLUSION

In conclusion, our result suggested that simultaneous screening of rhizobacteria for growth and yield promotion under pot and field experiment is a good tool to select effective PGPR for biofertilizer development biotechnology. PGPR are highly beneficial for plant growth and can serve as potential substitute for pesticides and chemical fertilizers. Even under unfavourable and stress conditions like UV-B exposure, PGPR can enhance seed germination and can exert a beneficial effect on plant growth.

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