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PLANT GROWTH PROMOTING ACTIVITY OF BACILLUS SPP. ON TURMERIC

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Abstract

In order to study the effect of plant growth promoting rhizobacteria (PGPR) on the growth and yield of turmeric, an experiment was conducted at Bhojia Institute of Life Sciences, India. Total five isolates, out of 50, belongs to Bacillus spp. (PGPR) designated as S1, S2, S3, S4, and S5 were successfully isolated and characterized on the basis of morphological and various biochemical methods. Subsequently, a pot experiment was conducted where turmeric plants are grown in sterilized and non-sterilized soil and mixed with isolates of PGPR to investigate the effect of bacterial isolates on the growth of experimental plant. The applications of bacterial strains increased the shoot length, leaf number, root length and root dry weight. Therefore, present study suggests that PGPR isolates viz. S1, S2, S3, S4 and S5 may be used as biofertilizers to enhance the growth and productivity of turmeric. Results of study showed seed Inoculation significantly enhanced seed germination and seedling vigour of turmeric. In pot experiment, shoot length and also root length and root dry weight significantly were increased by bacterial inoculation in both sterile and non-sterile soil. The results showed that inoculation with bacterial treatments had a more stimulating effect on growth and development of plants in non-sterile than sterile soil.

Keywords: Pot Experiment, PGPR, Rhizobacteria, Biofertilizer

1. INTRODUCTION

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the Ginger family *Zingiberaceae*. In Ayurvedic practices, turmeric is thought to have many medicinal and antibacterial properties with antiseptic effect for cuts, burns and bruises. It is also used for curing the problems of digestion, Liver Diseases, Cancer, Eye Disorder, Osteoarthritis, Atherosclerosis and Anemia. Thurmeric is also used in cosmetics for glow to the skin, make the skin fair, soft and smooth and spots caused due to pigmentation. Turmeric contains up to 5% essential oils and up to 3% curcumin, a polyphenol. Curcumin is also used as pH indicator. It shows yellow color below pH 7.4 and bright red above pH 8.6.

India is the largest producer, consumer and exporter of turmeric in the world. The global production of turmeric is around 11 lakh tonnes annually. India dominates the world production scenario contributing 78 % followed by China (8%), Myanmar (4%) and Nigeria and Bangala Desh together contributing to 6% of the global production.

Preparation of live microorganisms (bacteria, fungi) utilized for improving plant growth and crop productivity are referred to as biofertilizers or microbial inoculants [2, 18]. Biofertilizer is defined as a substance which, when applied to seed or plant surface or soil, promotes growth by increasing the supply or availability of primary nutrient to the host plant [20, 10, 11, 16, 8, 9]. Research in biofertilizers has resulted in the development of three kinds of microbial inoculants or biofertilizers including nitrogen fixing bacteria, phosphate solubilizing microorganisms, vesicular arbuscular mycorrhizae and plant growth promoting rhizobacteria (PGPR). Ninety five percent of Gram-positive soil *bacilli* belong to the genus *Bacillus*. The remaining 5% are confirmed to be *Arthrobacter* and *Frankia* [4, 19].

2. MATERIAL AND METHODS

Collection of soil sample and Isolation of Bacterial spp.

Soil sample was collected from the rhizospheric region (garden soil) of Bhojia Institute of Life Sciences, Budh, Baddi, Himachal Pradesh. Serial dilution agar plate technique was adopted for the isolation of Bacillus sp. and all isolates were biochemically characterized by Gram Staining, Indole production, Nitrate Reduction, Ammonia production, Phosphate solublization, HCN Production, Catalase Test, Endospore Staining, Motility Test, Meta-chromatic granule, Nitrate Reduction, starch hydrolysis, H2S production, citrate utilization, oxidation reaction, casein hydrolysis, 3-ketolactose production, urease production, lipolysis activity and gelatin liquefaction as described in Bergey's Manual of Determinative Bacteriology [7].

Antibacterial activity of Turmeric on isolated strains

To check the antibacterial activity of turmeric on isolated bacterial strains, Nutrient agar plates were used supplemented with turmeric powder. With the sterilized loop, selected bacterial isolates were streaked on the plates and incubate at 30^0C for 24 hours.

PGPR activity of isolates on turmeric

Earthen pots were used for conducting the pot experiments. These pots were filled with loam soil and sterilized to get control condition for conducting the experiment. Rhizomes of turmeric were surface sterilized in 1% $HgCl_2$ for 2 minutes and then, wash the rhizomes with sterilized distilled water at least 10 times to remove traces of toxic $HgCl_2$. These surface sterilized rhizomes were air dried in laminar air flow and then sown into sterilized pots (Four rhizomes per pot).

Bacillus strains along with their respective non-bacterized rhizomes as control were sown in following set of treatments:

Treatment 1- Sterilized soil + surface sterilized Rhizome (Standard)

Treatment 2- Soil + surface sterilized Rhizome (Standard)

Treatment 3- Sterilized soil + surface sterilized Rhizome coated with bacterial strain S1

Treatment 4- Soil + surface sterilized Rhizome coated with bacterial strain S1

Treatment 5 - Sterilized soil + surface sterilized Rhizome coated with bacterial strain S2

Treatment 6- Soil + surface sterilized Rhizome coated with bacterial strain S2

Treatment 7- Sterilized soil + surface sterilized Rhizome coated with bacterial strain S3

Treatment 8- Soil + surface sterilized Rhizome coated with bacterial strain S3

Treatment 9- Sterilized soil + surface sterilized Rhizome coated with bacterial strain S4

Treatment 10- Soil + surface sterilized Rhizome coated with bacterial strain S4

Treatment 11- Sterilized soil + surface sterilized Rhizome coated with bacterial strain S5

Treatment 12 – Soil + surface sterilized Rhizome coated with bacterial strain S5

1ml of log culture (10^8 cells) of each bacterial isolates was transferred as inoculum in the corresponding treatments. Treated and non-treated pots were irrigated with sterilized water daily. After every 7 days interval 2 ml of microorganism inoculum was inoculated in the corresponding pot as booster dose. Pots were irrigated daily. Different plant parameters like root length, shoot length, root dry weight, shoot dry weight and number of leaves were measured.

3. RESULTS AND DISCUSSIONS

All the isolated strains were Gram positive and morphologically rod-shaped. On biochemical characterization, it was observed that out of 50 isolates, 10 isolates show characteristics related to bacillus genus. Based on higher PGPR activity, five strains out of 10 bacillus spp. was considered for further research and labeled as S1, S2, S3, S4 and S5 (Table 1).

Table 1: Test for PGPR activity

Bacterial Strain	S1	S2	S3	S4	S5
Nitrate Reduction	+ve	+ve	+ve	+ve	+ve
HCN Production	+ve	+ve	+ve	+ve	-ve
Ammonia Production	+ve	+ve	+ve	+ve	+ve
Phosphate Solublization	-ve	-ve	-ve	-ve	-ve

Antibacterial activity of Turmeric

It has been observed that after 24hrs of incubation, turmeric was not shown any antibacterial activity on isolated bacterial spp. and all bacterial isolates able to grow on turmeric based medium.

PGPR activity on turmeric plant

Selected bacterial isolates were tested for their plant growth promoting activity with Turmeric. Result was recorded at different time intervals.

a. Result observed after 10 days

After 10 days of inoculation, it is observed that treatment 4 and12 showed increased shoot length 2cm both. While treatment 11 shown 1 cm shoot length in compariusion to controls. Same results also observed with other treatment except treatment 7, 9 and 10 where no growth was recorded (Figure 1).

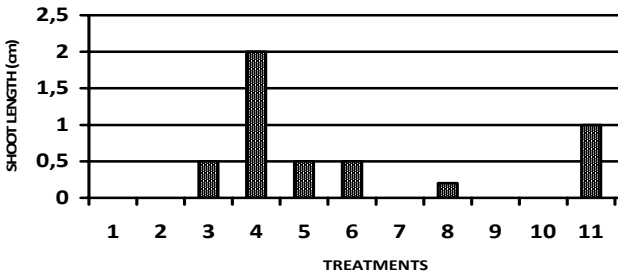

Figure 1: Effect of different PGPR strains on the plant growth of different treatments after 10 days

b. Result observed after 20 days

After 20 days of inoculation, it is interesting to note that controls show better result in compare to treatment 7 and 10. Instead of 7 and 10 treatments, others shows higher shoot length in comparison to controls as shown in Figure 2. Treatment 12 shown higher shoot length 16cm, while treatment 4, 14cm shoot length.

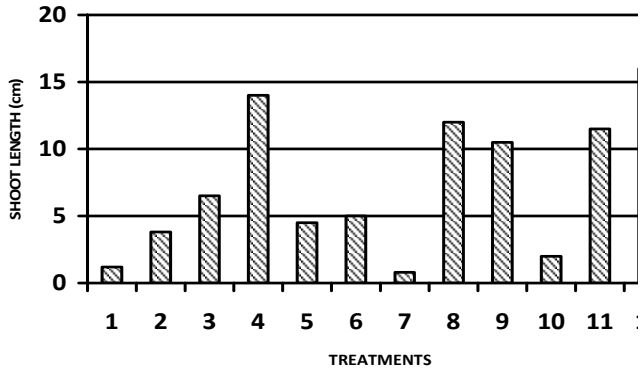

Figure 2: Effect of different PGPR strains on the plant growth of different treatments after 20 days.

c. Result observed after 30 days

On 30th day, all plants were harvested for analysis. In analysis, treatment 3, 4, 11 and 12 was recorded higher shoot length (approx. 22cm) in comparison to others. In all treatment (except controls) treatment 7 showed least increase in shoot length while treatment 5, 8, 9 and 10 showed significant shoot length (18, 18.5, 19.25 and 14.5 respectively) in comparison to control (Table 2). There is no significant difference was recorded between all treatments on the basis of number of leaves except treatment 7, where no leave was recorded (Table 2). The different treatments showed variation of root dry weight.

In all treatments, treatment 11 showed higher root dry weight (1.07gm) in comparison to other treatments and controls. Treatment 3,5,9 and 10 also showed significant root dry weight difference in comparison to controls.

Table 2: Effect of different isolated PGPR strains on the growth of turmeric after 30 days

Treatments	Shoot length (cm.)	Number of leaves	Root	
			Length (cm.)	Weight (gm.)
Treatment 1	8.7	1	3.0	0.02
Treatment 2	8.0	1.5	3.5	0.04
Treatment 3	21.20	1.5	6.25	0.44
Treatment 4	22.5	2	7.0	0.26
Treatment 5	18.0	1	4.5	0.30
Treatment 6	20.5	1	8.5	0.29
Treatment 7	7.0	-	1.0	0.09
Treatment 8	18.5	2	6.5	0.10
Treatment 9	19.25	2	7.0	0.35
Treatment 10	14.5	1	6.5	0.49
Treatment 11	21.5	2	7.5	1.07
Treatment 12	22.0	2	8.0	0.16

There were also significant differences in root lengths by the treatments.in all treatments , treatment 12, 11, 9 and 4 showed maximum root length, while other treatment's also showed significant increase in root length in comparison to controls except treatment 7 as shown in table 2.

4. CONCLUSIONS

This study was successful in selecting a range of rhizobacteria that showed plant growth promoting activities in turmeric in-vitro. The isolates were identified as belonging to Bacillus species. Among the fifty isolates, five isolates namely SP1, SP2, SP3, SP4 and SP5 was chosen for further studies on the basis of higher PGPR activity.

Plant growth promoting effects of PGPR strains in different crops were clearly demonstrated [21]. Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease [12]. This present investigation confirms the earlier works. It revealed that under *in vitro* conditions, inoculation with PGPR strains improved seed germination, shoot length, root length, leaf numbers and root dry weight over the control.

Similar improvement of plant growth by rhizobacteria has been reported in various plants by many reserchers [1, 8, 14, 15, 17].

Production of HCN, Ammonia and nitrate reduction are the most common mechanisms of action implicated in PGPR and indeed microbes demonstrating these attributes are widespread in rhizosphere [10, 13, 20]. Role of *Bacillus* spp. as successful PGPR has been well investigated [5]. The consistent performance of these PGPR strains *Bacillus* spp. indicates their potential to be used as commercial biofertilizer for the enhancement of growth and yield of Turmeric.

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