

Isolation and Characterization of PGPR from Paper Mill Effluent Infested Area

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ABSTRACT

Paper and pulp industry are considered as 17th most polluting industry due to its highly coloured and toxic wastewater discharge in the environment. A number of heavy metals get mixed within the agricultural soil through the paper mill discharge. These heavy metals are potent phytotoxic and they have huge negative impact on plant health. To minimize these impacts and to improve crop health deliberate application of chemical fertilizers is very common in present days but these chemical fertilizers are destroying the soil and plant health tremendously. In this situation rhizosphere researches suggest the concept of the application of PGPR. The group of root colonizing bacteria which enhance plant growth and development are called Plant Growth Promoting Rhizobacteria (PGPR). This group of bacteria improve plant's growth by direct as well as indirect mechanisms. These mechanisms involve IAA production, siderophore production, soil structure formation, decomposition of organic matter, solubilization of minerals, degrading organic pollutants, biocontrol of seed borne pathogens etc. In present study we have focused on isolation and characterization of PGPRs from paper mill effluent infested soil.

KEYWORDS: PGPR, heavy metals, paper industry, IAA, agrochemicals

INTRODUCTION

In the context of ever-increasing hunger of global population, the utilization of PGPR for minimizing the usage of agrochemicals is a potentially urgent issue [1]. PGPR can be defined as a group of beneficial bacterial strains which colonize the plant roots and stimulate plants growth and biocontrol potential. They enhance plant growth by promoting the plant-microbe symbiotic associations and by decreasing the activities of plant pathogens [3,4]. PGPR increases plant growth and biocontrol of seed borne pathogens by direct and indirect mechanisms. The direct mechanisms include solubilization of phosphates, fixation of nitrogen, IAA production, siderophore production and decreasing the ethylene level by ACC deaminase production etc [10,11]. The indirect mechanisms include bacterial, fungal and nematode pathogens suppression by production of different enzymes, such as, chitinase, protease, cellulases etc. Several other mechanisms of indirect plant growth improvement by PGPR include quorum sensing, signal interference, inhibition of biofilm synthesis, solubilization of mineral nutrients, systemic acquired resistance etc [5,6].

In recent years, industrialization got increased rapidly but the industrial activities accelerated the possibilities of environmental pollution also. Among different industries, papermill and pulp industries have huge drastic impact on agriculture and ultimately causing environmental degradation [12]. A huge amount of wastewater discharge from pulp and paper industries contain high amount of heavy metals as well as several toxic elements [7,9]. These toxic elements get accumulated in agricultural soils in

excessive amount in long-term use. Subsequently, these toxic heavy metals may lead to several physical problems to human bodies by entering through the food chain. Although the waste water discharge contain nitrates and phosphates but still it also contains heavy metals and other toxic materials which tremendously decrease the plant growth and crop yield. So, to minimize the toxic effects of the paper mill effluents, PGPR offers an attractive strategy. Hence, the present work was focused on to isolation and characterization of PGPR from rhizospheric soils of paper mill effluent infested area.

Materials and methods:

➤ Collection of soil sample:

Rhizospheric soil samples of brinjal (*Solanum melongena* L.) and cowbean (*Vigna unguiculata* (L.) Walp. plants were collected from different paper mill effluent infested areas of W.B. During collection upper 7 cm. soils were discarded and the rhizospheric bulk soil was collected by shaking procedure and subsequent brushing of root part of plants. The soil samples were aseptically collected in sterile polythene bags, brought to the laboratory and kept in aseptic condition as far as possible for further use.

➤ Isolation of bacteria:

Isolation of bacterial strains from collected soil samples were done by conventional serial dilution technique employing Nutrient Agar (NA) media (Beef extract- 3g; Peptone-5g; Agar-5g; Distilled water-1000ml., Sodium chloride- 5g, pH-6.8). The agar plates were incubated

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properly at 28° C for 48-72 hours. After that period the number of bacterial colonies developed on NA media were recorded and inoculated separately in NA slants. After that, those slants were maintained at 4° C for further work.

➤ Characterization of bacteria:

The characterization of the isolated bacterial strains was done by their morphological, staining and PGPR characteristics. Morphological characters include elevation, colour, edge of the colony. Gram nature determination of

each isolate was done by conventional Gram staining procedure. Among different biochemical tests, amylase test, catalase test, gelatin hydrolysis, urease test, citrate utilization, Voges Proskauer, methyl red was done based on conventional procedure of bacterial characterization (followed by Microbiology- A Laboratory Manual, 7th edition by Cappuccino Sherman) [8]. PGPR characterization was done by phosphate solubilization test, ammonia production, HCN production and IAA production tests.

➤ Results and Discussion:

The isolated bacterial strains were characterized according to morphological nature, staining properties, biochemical characters and on the basis of PGPR characters. All the results are depicted below:

Table 1: Microbial community of collected soil samples:

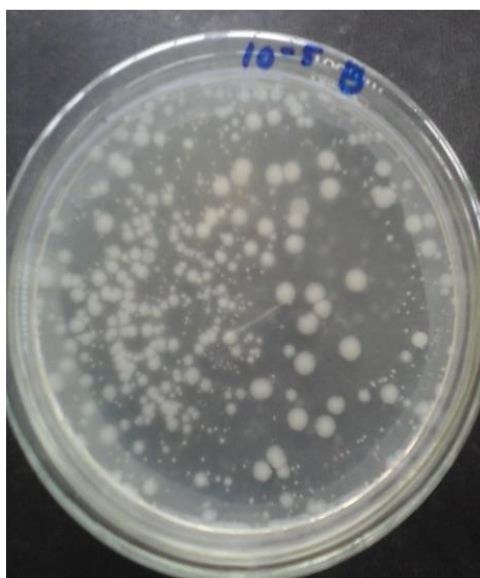
Soil Sample	Medium used	CFU (x 10 ⁶)	Bacterial Colony characters
A1	Nutrient Agar medium for bacterial culture.	42	Bacterial colonies are round, yellowish, smooth.
A2		27	Bacterial colonies are round, creamy, wavy.

Two soil samples were further assessed employing NA media for bacterial population. From the observed data it is quite clear that the bacterial colonies were nearly round, creamy to yellowish in colour.

Table 2: Physical characters of bacterial isolates:

Bacterial isolates	Colony Morphology				
	Colour	Form	Margin	Elevation	Gram nature
CPSR-1	Creamy-white	Punctiform	Entire	Flat	+
CPSR-2	Greyish-white	Rhizoidal	Undulate	Flat	+
CPSR-3	Moist-cream	Circular	Curled	Raised	+
CPSR-4	Glossy-white	Filamentous	Filamentous	Convex	-
CPSR-5	Glossy-white	Filamentous	Curled	Raised	+
CPSR-6	Moist-cream	Circular	Undulate	Flat	-
CPSR-6	Dark yellow	Rhizoidal	Filamentous	Convex	+
CPSR-7	Light yellow	Irregular	Erose	Raised	-
CPSR-9	Glossy-white	Rhizoidal	Curled	Raised	-
CPSR-10	Yellowish-white	Circular	Lobate	Flat	+

All the bacterial isolates collected from rhizospheric soil by conventional serial dilution technique were named after CPSR series (C= Chakdaha, P= PGPR, S= Soil, R=Root). All the bacterial colonies were characterized on the basis of the colony's nature, such as, colour, form, margin, elevation. The diversity in their colony's morphological nature indicated that every bacterial colony was different from each other.

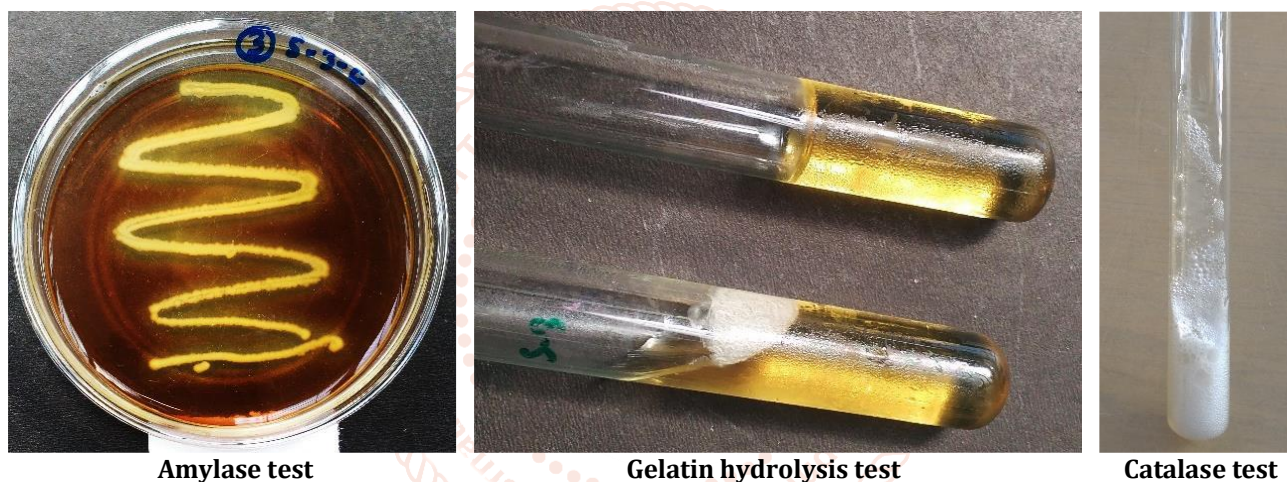


Photograph 1: Representative photographs of bacterial colonies grown on Nutrient Agar media.

Table 3: Biochemical characterization of bacterial isolates:

Bacterial isolates	Biochemical tests						
	Amylase ^a	Catalase ^b	Gelatin hydrolysis ^c	Methyl Red ^d	Voges Proskauer ^e	Citrate Utilization ^f	Urease ^g
CPSR-1	+	+	-	+	++	+	+
CPSR-2	+++	++	-	+	-	+	+
CPSR-3	+	+	-	-	+	-	+
CPSR-4	+	-	++	+	+	-	+
CPSR-5	+	-	+	+	++	+	+
CPSR-6	++	+	+	+	-	+	+
CPSR-6	+	+	+	-	+	+	++
CPSR-7	-	++	+	++	++	+	+
CPSR-9	+	+	+	-	++	-	+
CPSR-10	+	-	+	++	++	-	+

- a- Production of halo zone around the bacterial growth on Nutrient Agar medium (NA) indicated amyolytic activity.
- b- Production of bubbles in the bacterial culture indicated catalytic activity.
- c- Liquefaction of gelatin tube prior to refrigeration indicated hydrolysis of gelatin.
- d- Appearance of reddish colour above bacterial broth culture signified positive reaction.
- e- Colour of culture turns into red if the reaction becomes positive.
- f- Appearance of blue colour on culture medium indicated positive reaction.
- g- Colour of culture medium turns into pink during positive reaction.



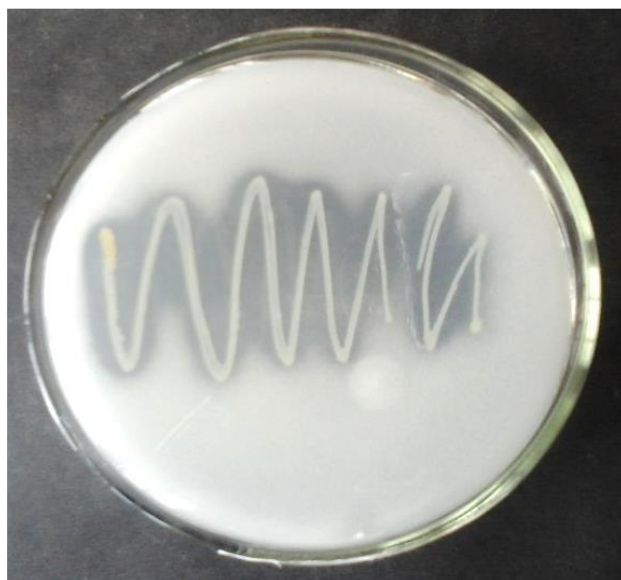
Photograph 2: Biochemical characterization of bacterial isolates

After biochemical characterization, the Plant Growth Promotion characters of those bacterial isolates were done. Among different tests, phosphate solubilization [8], ammonia production, HCN production, IAA production tests were done. The results are presented below:

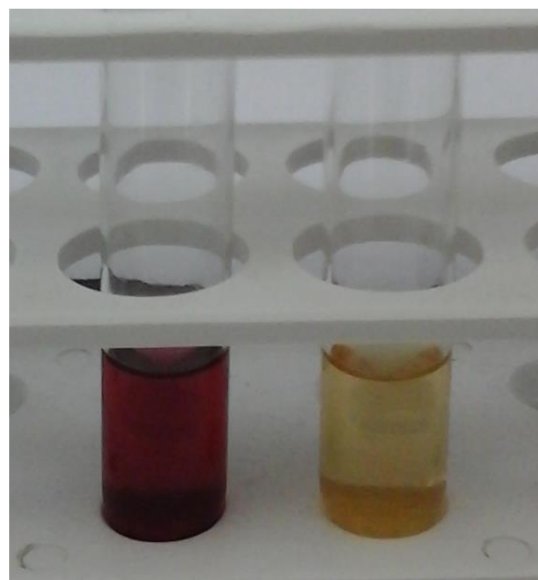
Table 3: Study on Plant Growth Promotion Activity of bacterial isolates:

Bacterial isolates	PGPR tests			
	Phosphate solubilization ^a	Ammonia production ^b	IAA production ^c	HCN production ^d
CPSR-1	+	++	++	-
CPSR-2	+	-	++	+
CPSR-3	+	+	+	-
CPSR-4	++	+	+	+
CPSR-5	-	+++	-	+
CPSR-6	+	+	+++	-
CPSR-6	+	+	+	++
CPSR-7	-	++	+	+
CPSR-9	-	+	+	-
CPSR-10	+	+	++	+

- “+” Sign indicates positive result and “-” sign indicates negative result. Number of + sign indicates the intensity of positive result.
- a- production of halo zone around the bacterial growth on Pikovskaya’s agar medium indicated positive result.
- b- Turning of colour of bacterial culture into fade yellow indicated ammonia production.
- c- Intensity of red colour of bacterial culture indicated the level of IAA production.
- d- Appearance of brown colour on filter paper indicated HCN production by bacterial isolates.



Phosphate solubilization test



IAA production test

Conclusion:

The results suggest that 10 bacterial isolates exhibited plant growth promoting activities. Therefore, these isolates can be applied as biofertilizers as they can enhance plant yield by different mechanisms under heavy metal stressed condition in paper mill effluent infested zone.

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