Impact of *Pseudomonas aeruginosa* MAJ PIA03 affecting the growth and phytonutrient production of castor, a primary host-plant of *Samia ricini*

Sosanka Protim Sandilya¹, Pinky Moni Bhuyan¹, Vijay Nageshappa¹, Dip Kumar Gogoi^{1*}, Devid Kardong²

¹Biotechnology Division, Central Muga Eri Research & Training Institute, Central Silk Board, Lahdoigarh, Jorhat-785700, Assam, India. ²Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India. *Corresponding author: gkdeep@rediffmail.com

Abstract

Castor (*Ricinus communis* L.) is a primary food plant of eri silkworm, *Samia ricini* Donovan endemic to North-east India. Plant growth promoting rhizobacteria (PGPR) plays crucial role in growth and productivity of agricultural crops. Eighty-five *Pseudomonas* strains were isolated from thirty rhizosphere soil samples of castor and their in vitro potentiality was screened for growth promoting ability and antifungal potentialities. Most of the selected *Pseudomonas* sp. produced indole acetic acid, gibberellic acid, ACC deaminase activity, ammonia, hydrogen cyanide and siderophore, when tested *in-vitro* for PGP activity. Besides, the strains MAJ PIA01, MAJ PIA03 and MAJ PIA24 have got antifungal activity against five common plant pathogens. Upon their formulation and application in field by curtailing the recommended dose of inorganic fertilizers to 50 %, isolate no. MAJ PIA03 efficiently enhanced the agronomical traits such as number of leaves per plant, leaf biomass, root length, stem base diameter, shoot length, root biomass and leaf moisture content. Isolate no. MAJ PIA03 has significant impact on leaf biochemical constituents and augments the nutritional quality of castor leaf. The most efficient isolate MAJ PIA03 was identified as *Pseudomonas aeruginosa* MAJ PIA03 by polyphasic approach and its 16S rRNA gene homology. The bio-formulation of *Pseudomonas aeruginosa* MAJ PIA03 with 50 % diminution on recommended inorganic NPK dose could be used as an effective mechanism to improve *Ricinus communis* plant growth as well as leaf nutritional enhancement for sustainable eri sericulture.

Keywords: Plant growth promoting rhizobacteria, *Pseudomonas* sp., *Ricinus communis*, *Samia ricini*, Sericulture

1. Introduction

Castor (*Ricinus communis* L.) belonging to the family Euphorbiaceae is an annual or perennial herb which is being utilized as a primary host/food plant by the multivoltine sericigenous insect *Samia ricini* Donovan (eri silkworm). Eri culture is a small scale textile industry endemic to the Northeastern states of India and has been a prime source of income among the people of this region since immemorial time (Sandilya et al., 2016). It is mainly concerned with the production of commercial silk and warm cloth and the production parameter lies mostly on the consumption of quality leaves. Sericulture farmers of Northeast region mostly opt for the inorganic fertilizers for years resulting in deterioration of agricultural soil quality. Since the yield reduction is a vital problem in organic farming (Lind et al., 2003), the integrated nutrient management (INM) with reduced chemical doses may boost the quality leaf yield for commercial silkworm rearing. Strategic implementation of INM practices lead to eco-friendly, sustainable and organic agricultural practice (Esitken et al., 2005).

Plant growth promoting rhizobacteria (PGPR) is a heterogeneous group of bacteria that originates mainly in the rhizosphere, at root surfaces or in symbiotic association with roots. Enormous diversity of rhizobacteria such as *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Clostridium*, *Enterobacter*, *Klebsiella*, *Phyllobacterium*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Vario-vovax*, *Xanthomonas*, etc. has been reported over the years (Glick et al., 2004). The impact of rhizobacteria on plant nutrition and growth enhancement is manifold. Multiple mechanisms of PGPR action could promote sustainable plant development as it has got direct effect on the enhancement of plant growth; either by synthesizing growth promoting substances, or facilitating nutrients uptake from environment (Viscardi et al., 2016).

Fluorescent *Pseudomonads* are aerobic, gram-negative bacteria, ubiquitous in agricultural soils and are well adapted to grow in the rhizosphere. *Pseudomonads* possess the ability to colonize and multiply in the rhizosphere and intercellular spaces of plant tissue and produce a wide spectrum of bioactive metabolites such as antibiotics, siderophore, HCN and growth promoting substances (Weller 2007). They can suppress pathogenic toxicity by producing specific antibiotics and siderophores to induce systemic resistance to the host (Dwivedi and Johri 2003). *Pseudomonads* also play a decisive role in plant growth promotion through reduction of ethylene production by synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase. The ACC deaminase enzyme modulates the level of ethylene by hydrolyzing ACC in ammonia and α -ketobutyrate (Marques et al., 2010). *Pseudomonas fluorescens* strain Psd solubilizes inorganic zinc and produces nitrate reductase along with certain antioxidant enzymes such as glutathione reductase and peroxidase which eventually supports the growth of wheat (Sirohi et al., 2015).

Reports on the exploitation of beneficial rhizobacteria for sustainable host plant cultivation in sericulture sector are very sporadic. Therefore, characterization and introduction of native potential Plant Growth Promoting Rhizobacteria (PGPR) may help in enrichment of soil nutrients and thereby increase quality castor leaf yield for eri silkworm rearing (Kumar et al., 2012). In the present study, an effort has been made to: (i) determine the quantitative growth promoting activity of *Pseudomonads*, isolated from castor rhizosphere, (ii) assess the impact of *Pseudomonads* affecting the growth and leaf biochemical constituents of the target crop and (iii) identify the most efficient isolate by polyphasic approach.

2. Materials and Methods

2.1. Isolation of pseudomonads

Pseudomonads were isolated from rhizosphere soil of castor plants collected from natural habitats of upper Assam, India. Samples were collected from Garmur satra (26.96873 oN 94.14370. oE), Kamalabari satra (26.94356 oN 94.20002 oE) and bank of river Brahmaputra (26.92356 oN 94.24210 oE) of Majuli river island, Assam with an elevation of 87.3, 89.2 and 84.3 m respectively. Sterile poly bags were used to carry the samples aseptically to the laboratory, further stored at 4 °C and processed within 24 hours for analysis. Isolation was carried out by serial dilution spread plate technique (Buchanan & Gibbon, 1974) using *Pseudomonas* Isolation Agar (PIA) medium (Hi-media Ltd., Mumbai). Soil sample was suspended in sterile saline solution (0.80 % NaCl) serially diluted up to 10^{-7} and inoculated on PIA medium at pH 6.8 and incubated at 32 °C (OVFU, O-CIS-4D). *Pseudomonas* colonies were selected based on the visual colony morphology and colours were further purified by subsequent culturing. Sub-cultured pure cultures were preserved at -80 °C in Luria Broth (LB) supplemented with 15 % glycerol for further study.

2.2. Morphological and biochemical characterization

Cultural characters of the pure isolates were studied on the basis of colony elevation, margin, form, texture and opacity. The morphological study was conducted by Gram staining microscopy method using Gram staining kit (Hi-media K001) (Holt *et al.*, 1994). The stained bacterial cells were observed under Phase contrast microscope (Olympus, CKX41) to monitor the Gram reaction, shape, orientation, etc. Biochemical characterization was carried out through culture dependent techniques as per the Bergey's Manual of

Determinative Bacteriology and using Biochemical Test Kit (Hi-media Ltd., Mumbai, India).

2.3. In vitro screening for PGP traits

2.3.1. Assay for Indole acetic acid (IAA)

IAA production by *Pseudomonas* isolates was estimated by little modification of the method described by Wohler (1997). The bacteria were cultured for 24 hours on nutrient broth and centrifuged at 14000 rpm for 5 minutes to collect the cells. The cell pellets were incubated at 37 °C for 24 h after suspending in 3.0 mL of phosphate buffer (pH 7.5) containing glucose (1%) and tryptophan (1%). After incubation, 2.0 mL of 5% trichloroacetic acid and 1.0 mL of 0.5 M CaCl_2 were added and filtered through Whatman paper no. 2. Salper solution (2.0 mL) was added to 3.0 mL of the filtrates taken in test tubes. The reaction mixture was then incubated at 28 °C for 30 minutes in dark condition. The absorbance was measured at 535 nm using UV-vis spectrophotometer (Systronics 2202) and concentration of IAA (mg L^{-1}) was calculated by calibrating with a standard curve made by using graded concentration of IAA.

2.3.2. Assay for Gibberellic acid (GA_3)

GA_3 production was measured as per Paleg (1965). 3.0 mL of culture filtrate obtained by the above method for IAA test was taken in test tube and added 2.0 mL of zinc acetate. Potassium ferrocyanide (2.0 mL) was then added after 2 minutes and centrifuged for 5 min at 1000 rpm. Finally, 5.0 mL of 30% HCl was added to 5.0 mL of the supernatant and incubated at 20 °C for 2 hours and the colour intensity of the samples was measured at 254 nm using uv-vis spectrophotometer. GA_3 concentration (mg L^{-1}) was determined by

calibrating with the standard curve of authentic GA₃ (Hi-media Ltd., Mumbai).

2.3.3. Assay for ACC deaminase

For ACC deaminase activity of the *Pseudomonas* isolates, the broth culture was centrifuged at 10,000 rpm for 5 min and the resultant pellets were suspended in 5.0 mL of 0.1 mol L⁻¹ of Tris HCl having pH 7.6. The solution is then centrifuged at 16,000 rpm for 5 minutes and the cell pellets were re-poised in 2.0 mL of 0.1 mol L⁻¹ Tris HCl (pH 8.5). The cell suspension was vortexed for 1 min after the addition of 30 µL of Toluene. Thereafter, 200 µL of the whole mixture were placed in microcentrifuge tubes and supplemented with 20 µL of 0.5 mol L⁻¹ 1-aminocyclopropane-1-carboxylate (ACC). The mixture was then vortexed and incubated for 15 min at 30 °C. After incubation, 1.0 mL of 0.56 mol L⁻¹ HCl was added to the solution and centrifuged at 16,000 rpm for 5 minutes at 32 °C. The resultant supernatant was then again mixed with 1.0 mL of 0.56 mol L⁻¹ HCl, then vortexed and added 2.0 mL of 2, 4-dinitrophenylhydrazine reagent and incubated at 30 °C for 30 minutes. The entire suspension was then boosted with 2.0 mL of 2 mol L⁻¹ NaOH and the absorbance was recorded at 540 nm. ACC deaminase activity was measured by calibrating with a standard curve prepared from serial concentrations of α-ketobutyrate (TCI Chemicals).

2.3.4. NH₃, HCN and Siderophore production

NH₃ production was determined by inoculating the pure *Pseudomonas* isolates into 10 mL of peptone water and incubated for 48 h at 32 °C. After incubation, 0.5 mL of Nessler's reagent was added to each tube. Appearance of yellow to brown colour indicates positive ammonia production by the isolates (Cappuccino and Sherman 1992).

2.3.5. Antifungal assay

Pseudomonas isolates were cultured on Nutrient broth medium for 48 h at 32 °C in orbital shaking incubator. The crude bioactive molecule was isolated from the culture broth by solvent extraction method using ethyl acetate in the ratio of 1:1 (Bordoloi et al., 2001). The solvent part was collected by using a separating funnel and evaporated *in-vacuo* to recover the crude extract. The crude extract was dissolved (0.5 mg mL⁻¹) in dimethyl sulfoxide (DMSO) and antifungal bioassay was carried out by Agar well diffusion method (Grammer 1976) against five plant pathogenic fungi viz, *Fusarium moniliformes*, *Fusarium oxysporum*, *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia solani* and *Fusarium glycopersicum*. The antifungal activity was evaluated by measuring the growth inhibition zone against test fungi.

2.4. Formulation of *pseudomonas* cultures

Based on the plant growth promoting efficiency, the *Pseudomonas* isolates were selected and mass cultured in nutrient broth for field experiment. Formulation of the *Pseudomonas* cultures has been prepared in carrier material, as in vermicompost and inert material (i.e., Charcoal) in 1:1 ratio with final microbial load of 108 colony forming units (CFU). Eight treatment combinations were formulated out of selected *Pseudomonas* strains for field experiment.

2.5. Cultivation of *R. communis* – experimental design

R. communis NBR-1 seeds were surface sterilized with 0.5 % (v/v) NaOCl for 10 minutes and washed with sterile distilled water. Treated seeds were sown in RBD (Randomized block design) on the experimental plot as per the recommendation of CMERTI, Central Silk Board, Ministry of Textiles, Govt. of

India, Assam (India). Input of recommended farm yard manure (FYM) i.e. NPK (urea: SSP: MOP = 13 g: 25 g: 3 g per plant) and organic manure i.e. cow-dung (1 kg plant⁻¹) was taken as control. The pits were inoculated with *Pseudomonas* sp. (8 strains) formulations (0.5 kg plant⁻¹) with 50% reduction in recommended NPK dose. Simultaneously, 0.5 kg plant⁻¹ of organic manure (cow-dung) was also supplemented in each pit. Castor plants were maintained through routine cultural operation during the growth period and harvesting occurred 14 weeks after the experimental cultivation.

2.6. Assessment of plant growth parameter

Different plant growth parameters such as number of leaves, leaf weight (fresh), stem base diameter, root length, leaf biomass, leaf moisture content and percentage of leaf biomass increase over control was studied to assess the most efficient treatment combination for growth of castor plant.

2.7. Assessment of Leaf biochemical constituents

Total carbohydrate content (%) was estimated by Anthrone method (Sadasivam and Manickam 2005). The method described by Lowry *et al.* (1951) was employed to determine the total soluble protein (%). Lipid content (%) of the leaf samples was ascertained by solvent extraction method using petroleum ether (Sadasivam and Manickam 2005). Phenol content was estimated by the method of Price and Butler (1975), whereas chlorophyll content (%) in castor leaves was resolved by following standard protocol (Arnon 1949). Crude fiber content was estimated as per the method of AOAC (Association of Official Agricultural Chemist, USA).

2.8. Molecular identification

Molecular identification of the most potential isolate was carried out by 16S rDNA homology analysis. Isolation of the genomic DNA of the potential *Pseudomonas* strain was done by standard methodology (Marmur 1961) by using Genomic DNA extraction kit manufactured by SRL Pvt. Ltd., Mumbai (India). The DNA purity and quantity were checked by spectrophotometer at 260 and 280 nm. PCR amplification of 16S rDNA was done with bacterial universal forward primer 16SF and reverse primer 16SR as described by Weisberg *et al.* (1991). The PCR for the 16S rRNA gene was performed with initial denaturation at 95 °C for 2 min followed by 35 cycles consisting of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, followed by a final extension of 5 min at 72 °C. The PCR products were purified by using a QIA quick PCR purification kit (Qiagen) according to the manufacturer's instructions. The partial sequencing of the 16S rDNA gene was carried out through the courtesy of DNA sequencing service, Merck Millipore (Bangalore GeniTM), Bangalore, India by using the same primer. The partial 16S rDNA gene sequence was aligned against representative reference sequences of the most closely related members obtained from the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov/BLAST>). A neighbor joining phylogenetic tree was constructed in bioinformatics software programme MEGA 6.0 by multiple alignments using CLUSTALW (Saitou and Nie 1987). The identified gene sequence was submitted to NCBI GenBank and accession number was obtained.

2.9. Statistical analysis

The field experiment was designed in RBD with eight different treatments of *Pseudomonas* sps. and controls. Data were recorded as arithmetic mean of three

independent replications for each treatment. Statistical analysis was performed by using the SPSS programme, version 16.0 through analysis of variance (ANOVA). Statistical significance of differences ($p < 0.05$ and $p < 0.01$) was determined by Duncan's multiple range test (DMRT). Standard error means (SEM), critical difference (CD at 5% level) and correlations were performed with different variables and Spearman's correlation coefficients were determined.

3. Results

3.1. Isolation and characterization

Eighty five pure culture of rhizobacteria were isolated from the thirty soil samples collected from various localities. Total viable colony count of *Pseudomonas* sp

on PIA medium was highest (2.4×10^3 CFU/mL) in the soil samples of Garmur satra, Majuli, Assam.

All the isolates were found to be gram negative rod of different sizes and texture. Characterization of the isolates resulted varied biochemical properties as per Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

3.2. Microbial screening and bio-assay

Based on the qualitative screening for plant growth promoting (PGP) activity and antifungal property, eight *Pseudomonas* isolates were selected for further analysis. Qualitative screening revealed that six *Pseudomonas* isolates have got HCN production ability and all the isolates synthesized NH_3 *in-vitro*, whereas most of the *Pseudomonads* except MAJ PIA02 and MAJ PIA66 were capable of siderophore production (Table 1).

Table 1. Production of NH_3 , HCN, Siderophore and antifungal activity of *Pseudomonads* against five plant pathogens

Pseudomonas isolates	NH_3 production	HCN production	Siderophore production	Inhibition zone diameter (mm)				
				<i>F.</i>	<i>F.</i>	<i>F.</i>	<i>R.</i>	<i>F.</i>
				<i>moniliformes</i>	<i>oxysporum</i>	<i>oxysporum</i>	<i>solani</i>	<i>oxysporum</i>
					NCIM 1281	f. Ciceri		<i>lycopersicum</i>
Control	NA	NA	NA	NA	NA	NA	NA	NA
MAJ PIA01	+	+	+	13	11	09	12	14
MAJ PIA02	+	NA	NA	NA	15	NA	NA	19
MAJ PIA03	+	+	+	26	35	31	28	30
MAJ PIA24	+	+	+	18	20	14	16	15
MAJ PIA43	+	+	+	10	NA	NA	15	12
MAJ PIA66	+	NA	+	11	12	NA	16	09
MAJ PIA76	+	+	+	18	NA	18	NA	10
MAJ PIA79	+	+	NA	21	NA	NA	15	NA

*NA: No activity; '+': Positive activity; Numericals: Inhibition zone diameter in mm

In-vitro antimicrobial bioassay result showed that MAJ PIA03 has produced inhibition zone diameter against five common plant pathogens viz., *Fusarium monoliformes* (26 mm), *Fusarium oxysporum* (35 mm), *Fusarium oxysporum* Ciceri (31 mm), *Rhizoctonia solani* (28 mm) and *Fusarium glycopersicum* (30 mm) (Table 1).

IAA production by the *Pseudomonas* isolates was ranged between 5.19 ± 1.29 mg/L to 27.84 ± 0.52 mg/L. Highest amount of IAA was produced by MAJ PIA03 (27.84 ± 0.52 mg/L) whereas, lowest was recorded in MAJ PIA79 (5.19 ± 1.29 mg/L). Production of IAA among the eight *Pseudomonas* isolates could be translated by the expression MAJ PIA03>MAJ PIA76>MAJ PIA24>MAJ PIA66>MAJ PIA43>MAJ PIA02>MAJ PIA01>MAJ PIA79 (Figure 1). Similarly, MAJ PIA03 synthesized maximum (8.21 ± 0.15 mg/L) amount of GA₃ and it was minimum in MAJ

PIA02 (1.3 ± 0.23 mg/L). Isolate no. MAJ PIA76 (5.88 ± 0.14 mg/L) showed substantial production of GA₃ as compared to MAJ PIA03 (Figure 1). The translation of GA₃ production by the *Pseudomonads* could be expressed as MAJ PIA03>MAJ PIA76>MAJ PIA79>MAJ PIA01>MAJ PIA66>MAJ PIA43>MAJ PIA24>MAJ PIA02.

Out of the eight *Pseudomonads*, six isolates have the ability to degrade ACC into α -ketobutyrate. Highest ACC deaminase activity ($0.84 \mu\text{g}/10\text{mL}/\text{h}$) was recorded in MAJ PIA03, followed by MAJ PIA01 ($0.66 \mu\text{g}/10\text{mL}/\text{h}$) (Figure 2). Castor leaf and root biomass is positively correlated with synthesis of IAA, GA₃ and ACC deaminase by *Pseudomonads*. Enhancement of these plant traits, particularly leaf biomass is significantly correlated ($p < 0.05$) with the production of IAA. On the contrary, leaf biomass was negatively correlated to ACC deaminase activity of the isolates.

Figure 1. Production of IAA and GA₃ (mg/l) by *Pseudomonas* isolates.

Figure 2. ACC deaminase activity ($\mu\text{g}/10\text{ml}/\text{min.}$) of the *Pseudomonas* isolates.

3.3. Growth parameter analysis

Field trial under optimum growing conditions was conducted and significant enhancement ($P < 0.05$) in growth and yield over control was recorded in the castor plants treated with *Pseudomonas* isolates and 50% reduced dose of inorganic NPK. Highest leaf number (49.40 ± 7.28 nos.), fresh leaf weight (396 ± 63.80 g), root length (59.60 ± 1.92 cm), root biomass (55.10 ± 11.24 g), plant length (260 ± 19 cm), leaf biomass (97.60 ± 5.47 g) and leaf moisture content (354.90 ± 51.13 g) was recorded in treatment T₅.

The shoot elongation ranged from a minimum 1.72 m (T₄) to a maximum of 2.60 m when inoculated with T₆. Similarly, root length of the plants were varied from 30.40 cm (T₃) to 59.60 cm (T₅) and most of the treatment promoted root growth significantly ($P < 0.05$) in comparison to untreated plants with an exception to T₂, T₃, T₇ and T₉ (Table 2).

Result indicated significant increase in shoot length (260 ± 19 cm) over control in treatment T₆, whereas, biomass per leaf increase over control (47.65 %) was found maximum in treatment T₅. The average percentage biomass increase was 47.65 %, 38.57 % and 26.77 % when isolate nos. MAJ PIA03, MAJ PIA66 and MAJ PIA24 were applied, respectively (Table 2). Similarly, significant difference was observed in stem base diameter of castor treated with different combinations in comparison to control. The critical differences, standard error and significance at different confidence level among the variables are mentioned in Table 2.

Inoculation of *Pseudomonads* treatment combinations influenced on the root and leaf biomass production of *R. communis*. The leaf dry biomass of *R. communis* varied from 60.40 g to 97.60 g where all the treatment combinations contributed significantly ($p < 0.05$) against the control. However, treatment T₅ (97.60 g) and T₈ (91.60 g) showed maximum leaf biomass in comparison to the other treatment combinations (Table 2).

Table 2. Growth parameter of the castor plants supplemented with different *Pseudomonas* treatment combinations.

Treatments	No. of leaves per plant	Fresh leaf weight (g)	Stem base diameter (cm)	Root length (cm)	Root biomass (g)	Shoot length (cm)	Leaf biomass (g)	Leaf moisture content (g)	Percentage of leaf biomass increase over control (%)
T ₁ (FYM-inorganic)	42.80**	291.50**	3.56**	41.80**	48.10**	240 ^{ns}	66.10**	272.70*	NA
T ₂ (Cow-dung)	43.70**	357.00**	3.08**	35.50**	43.00**	216 ^{ns}	66.90**	281.90*	01.21
T ₃ (50% FYM + MAJ PIA01)	42.15**	254.00**	2.79**	30.40**	44.50**	202 ^{ns}	74.50**	232.80*	12.70
T ₄ (50% FYM + MAJ PIA02)	42.23**	305.00**	2.20**	54.90**	46.70**	172 ^{ns}	74.00**	255.80*	14.97
T ₅ (50% FYM + MAJ PIA03)	49.40**	396.00**	2.46**	59.60**	55.10**	175 ^{ns}	97.60**	354.90*	47.65
T ₆ (50% FYM + MAJ PIA24)	38.56**	321.00**	3.94**	57.20**	49.30**	260 ^{ns}	83.80**	300.40*	26.77
T ₇ (50% FYM + MAJ PIA43)	34.75**	248.00**	3.07**	31.80**	51.21**	200 ^{ns}	82.40**	231.80*	24.65
T ₈ (50% FYM + MAJ PIA66)	31.69**	219.00**	3.24**	51.80**	42.00**	229 ^{ns}	91.60**	189.40*	38.57
T ₉ (50% FYM + MAJ PIA76)	44.92**	330.00**	2.17**	40.40**	37.86**	236 ^{ns}	73.20**	292.80*	10.74
T ₁₀ (50% FYM + MAJ PIA79)	45.34**	345.00**	3.87**	56.60**	40.22**	230 ^{ns}	60.40**	325.60*	-08.62
S. Em (±)	2.74	25.82	0.28	3.17	2.47	24	2.68	28.97	-
S. Ed (±)	3.87	36.52	0.40	4.49	3.49	35	3.79	40.98	-
C.D 5% (±)	8.09	76.17	0.84	9.37	7.29	73	7.91	85.48	-

*NA: Not applicable, S. Em: Standard Error Mean, S. Ed: Standard Error Deviation, C. D: Critical Difference, **: $p < 0.05$, *: $p < 0.01$ and ns: non-significant

3.4. Leaf biochemical characterization

Among the five essential leaf biochemical components, total carbohydrate (33.16 ± 0.85 %), phenol (25.81 ± 0.45 %) and lipid (25.52 ± 1.13 %) content was found maximum in the leaf samples of treatment T₅ (Figure 3 & 4). Similarly, protein (16.04 ± 0.65 %) content was highest in T₆, which has been followed by T₅ (15.09 ± 0.64 %). Moreover, T₅ enhanced the Chlorophyll 'a' (24.87 ± 0.77 mg/g tissue), chlorophyll 'b' (20.53 ± 0.72 mg/g tissue) and total chlorophyll (20.15 ± 1.17 mg/g tissue) contents of castor plant over other treatments (Figure 5). Nitrogen content and crude fiber content was estimated highest in T₈ (1.96 ± 0.07 %) and T₅ (9.78 ± 0.71 %), respectively which has been followed by T₆ (1.86 ± 0.07 % and 8.85 ± 0.06 %) (Figure 6).

3.5. Molecular identification

Based on the above results, the most efficient *Pseudomonas* isolate MAJ PIA03 was identified upto species level through, morphological, biochemical (Table 3) and 16S rRNA gene homology. The 16S rDNA sequence has been obtained through the courtesy to Merck Milipore (Bangalore Genei), Bangalore, India. The sequence has been aligned with the closest homology by using the BLAST tool of NCBI (National Centre for Biotechnology Information). The phylogenetic tree showed closest homology of the strain with *Pseudomonas aeruginosa* NBRC 12689 and hence identified as *Pseudomonas aeruginosa* strain MAJ PIA03 (Figure 7). The 16S rDNA sequence was submitted to NCBI and obtained the accession no. KM875456.

Figure 3. Total carbohydrate and lipid content (%) of castor leaf treated with *Pseudomonas* isolates.

Figure 4. Protein and phenol content (%) of castor leaf treated with *Pseudomonas* isolates.

Figure 5. Chlorophyll content (mg/g tissue) of castor leaf treated with *Pseudomonas* isolates.

Figure 6. Nitrogen and crude fiber content (%) of castor leaf treated with *Pseudomonas* isolates

Table 3. Morphological and biochemical characterization of the *Pseudomonas aeruginosa* MAJ PIA03

Characteristic features	Result	Characteristic features	Result
Gram reaction	Negative	<i>Sugar utilization test</i>	
Morphology	Rod	Glucose	+
Methyl red test	+	Sucrose	+
Indole test	-	Lactose	-
Citrate utilization	+	Fructose	+
Catalase test	+	Mannitol	+
Nitrate reductase	+	Arabinose	-
Voges Proskauer	-	Trehalose	-
ONPG	-	Sorbitol	-
Gelatin liquefaction	+	Adonitol	-
Phenylalanine	-	Cellobiose	-
Starch hydrolysis	-	Iso-propanol	-
H ₂ S production	-	Maltose	-
Arginine	+	Methanol	-
Ornithine	+	Galactose	-
Succinate	+	Mannose	-
Starch	-	Rhamnose	-
Tryptophan	-	Xylose	-
Urease	+		
Oxidase	+		
Lipase	+		
Alanine	+		
Lactate	+		
Malate	+		
Gluconate	+		
Glycerate	+		
Malonate	+		
Benzylamine	-		

* '+': Positive activity, '-': Negative activity

Figure 7. Phylogenetic tree-based on the 16S rDNA sequence indicating the position of strain MAJ PIA03 (using Neighbor Joining method).

4. Discussion

Pseudomonads are the first commercially used microbes isolated from soil as a biological means of plant growth promotion (Reed and Glick 2004). In this study, out of all *Pseudomonads*, isolate no. MAJ PIA03 produced highest amount of IAA, GA₃ and ACC deaminase *in vitro*. Most of them have synthesized some agriculturally important compounds such as HCN, NH₃, siderophore, antifungal agents, etc. and can be utilized in sustainable castor cultivation in ericulture. Bholay *et al.* (2012) observed biosynthesis of pyoverdine by *Pseudomonas aeruginosa* which acts as a siderophore. PGPR produce NH₃, HCN and Siderophore which enhance nitrogen accumulation, plant biomass and some important traits such as root and shoots elongation in agricultural crops (Marques *et al.*, 2010). Kumar *et al.* (2012) reported on production of IAA by *Pseudomonas* sp. FBK3 (21.31 mg/L), which is slightly lower than *Pseudomonas* sp. MAJ PIA03 (27.84 mg/L), used in this study. Inoculation of other PGPR such as *Bacillus* sp. OSU142 and *Bacillus* sp.M3 in sugar beet, barley corn (Ataoglu *et al.*, 2004) and tomato (Turan *et al.*, 2004) stimulated various growth parameters. Similarly, application of *Bacillus* sp.WG4 effectively protect ginger rhi-

zomes from being attacked by *Pythium myriotylum* by secreting antifungal metabolite pyrrole [1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(phenylmethyl) (Jimtha *et al.*, 2016).

In-vitro results of PGPR species may not be always reproduced under field, semi-field or even greenhouse conditions as the interaction between the associative PGPR and plants could be unstable (Marques *et al.*, 2010; Zhender *et al.*, 1999). They further stated that, performance of PGPR is directly correlated to different environmental conditions along with the soil characteristics. This study established the efficiency of the *Pseudomonads* prevalent to castor rhizosphere by enhancing up to 47.65% of leaf biomass over control under field condition. Marques *et al.* (2010) reported that *Sphingobacterium* sp. 1ZP4, *Bacillus* sp., *Achromobacter* sp. and *Ralstonia* sp1C2 influence on root, shoot elongation and biomass production of *Zea mays* in greenhouse condition. Similarly, *Pseudomonas putida*, *Azospirillum lipoferum*, *Gluconacetobacter azotocaptans*, *Azospirillum zeae* and *Azospirillum canadense* were reported to enhance growth of agricultural crops by synthesizing PGP agents (Mehnaz 2007b). Firmicute strain *Bacillus subtilis* (Araujo 2008) is an efficient plant growth promoter in *Zea mays* and highly effective against tomato wilt disease.

Leaf biomass and growth was enhanced significantly, in plants treated with *Pseudomonas* sp. MAJ PIA03 and 50 % supplementary NPK over recommended dose. Application of native *Pseudomonads* on castor plant as PGPR may induce systemic resistance against soil born diseases. Leaf biomass and stem diameter was also increased by applying *Pseudomonas* fluorescence Pf5 in high bush blue berry (De Silva *et al.*, 2000).

Moderate range of phenol content was found in leaves treated with T5, however, high phenol content affects on the feeding efficiency of silkworm (Rao *et al.*, 2009). Total phenol content and insect resistance have a positive association with each other where the former plays a crucial role in plant defence mechanism against entomo-pathogens (Anjani *et al.*, 2010). Larval weight and cocoon weight are influenced by the nitrogen and crude protein content of castor leaves (Sarmah *et al.*, 2011). Besides, Akhtar *et al.* (2011) observed significant rise of chlorophyll content and other growth parameter such as shoot length, root length, shoot fresh and dry weights, root fresh and dry weights and number of nodules per plant in *P. fluorescence* treated plants against control. Prathibha and Siddalingeshwara (2013), noticed augmentation of total carbohydrate and total soluble protein content during application of *Bacillus subtilis*, *P. fluorescence* on seeds of sorghum plant. Application of NO_3^- , NH_4^+ or NO_3NH_4 singly or in combination with PGPR inoculation led to a significant increase in root dry weight, shoot dry weight, leaf number, shoot length, stem base diameter, crude fiber and nutrient uptake (Tchiazé *et al.*, 2016).

This study reflects the role of native *Pseudomonas* on castor plant as PGPR on upgrading the nutritional quality of leaf. Leaf nutritional components *viz.* carbohydrate, protein, lipid, fat, chlorophylls, fiber etc., play significant role on growth and productivity of *S. ricini*.

5. Conclusions

The sericulture farmers of Northeast India use considerably large amount of inorganic fertilizer to replenish soil N, P and K resulting high cost and increased environmental pollution. Inoculation with native *Pseudomonads* have significant contribution to agro-ecosystems by N_2 fixation, P-solubilization, synthesis of biocontrol agent and PGP compounds such as IAA, GA₃, ACC deaminase, NH_3 etc. Although, numerous *Pseudomonas* species have been isolated till date from different agricultural localities but using native rhizosphere isolates is an added advantage for better adaptation. Application of *Pseudomonas aeruginosa* MAJ PIA03 with 50% restrain on inorganic NPK dose promoted root biomass by 12.70% and leaf biomass by 32.27% over control. The phytochemical constituents such as total chlorophyll, protein, carbohydrate, crude fiber and lipid content were increased in castor leaf while treated with *Pseudomonas aeruginosa* MAJ PIA03. Bio-formulation of *P. aeruginosa* MAJ PIA03 with reduced inorganic NPK can be a potential INM package in future for sustainable cultivation of *R. communis* for eri sericulture industry.

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