High yield and quality silk fibre production by muga silkworm, *Antheraea assama* through application of plant growth promoting rhizobacteria

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Plant growth promoting rhizobacteria (PGPR) were isolated from the rhizosphere of som plants (*Machilus bombycina*) maintained at the experimental farms of Central Muga Eri Research and Training Institute, Lahdoigarh, Jorhat. Microbial isolates were characterized and screened for effectiveness by spraying combinations of strains on the som plants. Selective and specific functional compatibility relationships in plant response between the microbial inoculants were observed. The effects of the PGPR combinations through increase in chlorophyll content, free amino acid, total protein, reducing sugar, carbohydrate and dry weight were studied. Five strains showing growth-promoting activity were selected and all the combinations had positive effect on the biochemical parameters studied, but the combination of RB1 + RB3 + RB4 + RB5 + RB8 strains produced the best result. Muga silkworm larvae fed on som leaves of the plant treated with this strain combination had higher activity of the enzymes, viz. trehalase, transaminase and phosphorylase in the silk gland, haemolymph and fat body. The cocoons of these silkworms produced more silk in terms of quality and quantity. This study could be exploited for improvement in quality and quantity of silk production through the application of PGPR.

Keywords: *Antheraea assama*, cocoon, *Machilus bombycina*, plant growth promoting rhizobacteria.

THE application of plant growth promoting rhizobacteria (PGPR) that colonizes the rhizosphere results in modification of plant health and development[1]. The properties of PGPR offer great promise for agronomic application. Recent progress in understanding their diversity, colonizing ability, mode of action, formulation and application has facilitated their development as reliable components in the management of sustainable agricultural system. Although significant control of plant pathogens or direct enhancement of plant development has been demonstrated by PGPR in the laboratory and greenhouse, results in the field have been less consistent[2].

Since the association between som plants (*Machilus bombycina*) and rhizobacteria has not been studied so far, here an attempt has been made to determine if well-characterized PGPR strains which demonstrate growth promotion in other plants, also enhance plant growth in som. *M. bombycina*, family Lauraceae, is one of the main primary host plants of muga silkworm (*Antheraea assama*), and has significant effects on their health and survival. Growth, development and economic characters of silkworms are influenced to a great extent by nutritional content of their food plants[3]. The aim of the present study was to investigate the effects of spraying PGPR strains isolated from the vicinity of the rhizosphere of som plants and to test whether this can improve the physiological and biochemical potential of the crop, which in turn will give a good source of food material for the muga silkworm larvae for better silk production.

Soil samples were collected from the rhizosphere of som (*M. bombycina* King), from the muga food plantation area of Central Muga Eri Research and Training Institute (CMERTI), Lahdoigarh, Jorhat. A total of 45 soil samples were collected. Each soil sample was examined for pH and urease activity. The pH of the soil was found to be acidic. Isolation and identification of microorganisms was done according to Cappucino and Sherman[4], and *Bergey's Manual*[5] on five different media, viz. nutrient agar (g/l: peptic digest of animal tissue 5, beef extract 1.50, yeast extract 1.50, sodium chloride 5, agar 15); potato dextrose agar (PDA, g/l: potato infusion 200, dextrose 20, agar 15), King agar B (g/l: agar 10, dipotassium hydrogen phosphate 1.5, magnesium sulphate 1.5, mixed peptone 20), yeast malt agar (g/l: peptic digest of animal tissue 5, yeast extract 3, malt extract 3, dextrose 10, agar 20) and actinomyces agar (g/l: agar 15, L-asparagine 0.1, dipotassium phosphate 0.5, ferrous sulphate 0.001, magnesium sulphate 0.1, sodium caseinate 2, sodium propionate 4).

The test fungal and bacterial plant pathogens were *Pestalotiopsis disseminate* (collected from CMERTI), *Fomes lamoensis*, *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum*, *F. solani*, *Aspergillus niger*, *Rhizoctonia solani* (obtained from Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi).

Line inoculation was done on PDA[6]. For this, an actively growing mycelial disc (6 mm) of plant pathogen was placed on one side of the petri plate 2 cm inside the periphery and a loopful of rhizobacterial strain streaked in a line on the opposite side at a distance of 5 cm from the mycelial disc. The plates were incubated at 28 ± 2°C and inhibition zones were measured as the distance (in cm) between the rhizobacterial test antagonist and test fungal pathogen after seven days of growth.

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For spot inoculation, an actively growing mycelial disc (6 mm) of plant pathogen was inoculated at the centre and a loopful of the rhizobacterial strain was inoculated on the PDA plate 2 cm inside the periphery at the two opposite equidistant sides and incubated at 28 ± 2°C. Inhibition zone was measured as the distance (in cm) between the rhizobacterial test antagonist and test fungal pathogen after seven days of growth. PDA plate inoculated with mycelia of plant pathogen was taken as control[7].

To monitor root colonization by rhizobacteria, the antibiotic-resistant strains of RB1 (ampicillin, 100 mg/l), RB3 (ampicillin, 110 mg/l), RB4 (ampicillin, 50 mg/l), RB5 (ampicillin + rifampicin, 100 mg/l) and RB8 (rifampicin, 50 mg/l) were used. For this, a heavy inoculum of the strain was streaked on nutrient agar (NA) and actinomycetes agar media containing the respective antibiotics. Drug resistance against the antibiotic was evaluated.

Root colonization monitoring was done according to Dileep Kumar and co-workers[8,9], by growing the isolates in nutrient broth (NB) and actinomycetes broth respectively. The som plants were surface-sterilized in 2.4% sodium hypochlorite solution for 3 min followed by rinsing in 3% hydrogen peroxide for half an hour and then potted in sterile soil till the roots were established. The broth containing the bacteria of interest was mixed with sterile moist soil to make a slurry. Saplings of som were dipped for 12 h in the slurry till approximately 5 g of the slurry had been attached to the roots; the som was planted in a pot containing sterile soil. Saplings were sampled for root colonization by the introduced bacteria after 10, 20 and 30 days. This was done by cutting 1 g of fresh root into 1 cm segments from bacterized plants and keeping them in 5 ml sterile distilled water and shaking for 3–4 min to release the rhizoplane bacteria into the water. Appropriate dilutions of the bacterial suspensions were pour-plated on NA and actinomyces agar containing ampicillin and rifampicin to evaluate the introduced bacteria. NA without any antibiotic was used to evaluate the total aerobic bacteria. The CFU/g of the introduced and total aerobic bacteria was counted after 2 days of growth at 28 ± 2°C.

Isolated rhizobacterial strains were grown on NA and actinomyces agar media. The strains were identified at Microbial Type Culture Collection and GeneBank (MTCC), IMTECH, Chandigarh.

For growth-promotion activity of five selected rhizobacterial strains, 3–4-month-old potted som plants were taken as the test crop[10–12]. For inoculum preparation four strains (RB1, RB4, RB5, RB8) were grown in NB and one *Actinomyces* strain (RB3) was grown in the Actinomyces Broth. The bacterial culture (25 ml each) was sprayed individually near the root region of the respective potted plant. The measure of inoculum or number of bacterial cells used for spraying was calculated by taking the absorbance at 600 nm. The OD (optical density) of the overnight-grown bacterial culture was measured at 600 nm and was found to be 0.5, which is almost equivalent to 1.2×10^7 CFU/ml. Selection of successful rhizobacterial strains or combinations for field trials was conducted at the North-East Institute of Science and Technology (NEIST; formerly RRL), Jorhat based on the enhancement of morphological characters in terms of increase in shoot height and increment in leaves after 90 days of treatment of the potted plants. Three treatments, viz. (RB1 + RB3 + RB5), (RB4 + RB8) and (RB1 + RB3 + RB4 + RB5 + RB8) were randomly selected for field trials at NEIST. A 20% dilution of the bacterial consortium bioformulation was sprayed on leaves, stems and near the collar region of the som plants.

For the field experiment we chose the experimental som plantation farm of the CMERTI. Biochemical studies with the young, tender and mature, disease-free leaves were performed. Young, tender leaf samples (0.1 g) were collected on the fourth, 12th, 15th and 30th day after treatment with PGPR and mature leaf samples (0.1 g) were collected on the 60th day after spraying. They were subjected to biochemical estimation. Total chlorophyll content was estimated by AOAC method[13], total carbohydrate content was estimated by the method of Hedge and Hofreiter[14], reducing sugars by the method of Somogyi[15], total soluble protein by the Folin Ciocalteau method as modified by Lowry *et al.*[16] and free amino acids by Lee and Takahashi[17]. SDS–PAGE was carried out according to Laemmeli[18].

The fifth instar larvae were subjected to enzyme assay studies after being fed with the som leaves treated with PGPR. Three tissues, viz. silk gland, haemolymph and fat bodies were isolated for the study. The activity of trehalase was determined by the method of Dahlquist[19], and Derr and Randall[20], by measuring the rate of reducing sugars released from trehalose by the enzyme source. The phosphorylase activity was assayed by the method of Green and Stumpf[21], as modified by Srivastava and Krishnan[22], by estimating the inorganic phosphate (Pi) released, which was quantified by the method of Fiske and Subba Row[23]. The transaminase activity was assayed following the method of Bergmeyer and Bert[24], by studying the amount of pyruvate released per milligram of protein.

Sericin was extracted from the muga silkworm cocoon using the method of Akiyama *et al.*[25]. Silk fibre analysis was carried out according to Krishnaswami *et al.*[26].

Statistical analysis was done using analysis of variance (ANOVA); critical difference of the treatment significance was calculated at $P < 0.05$. Results are means of three experiments each.

Among the 120 rhizobacterial strains isolated from 45 soil samples tested for antimicrobial property against some plant pathogens, five strains have shown inhibition against the tested pathogens. Results show that RB1, RB3 and RB4 strains have good inhibition zone against *P. disseminata*, the fungal pathogen of som plant (Table 1). RB1 and RB4 have inhibition zone against all the plant pathogens tested.

Table 1. *In vitro* antibiosis study of different rhizobacterial strains against several plant pathogens (fungal/bacterial strains)

Fungal/bacterial strains	Inhibition zone (cm)				
	RB1	RB3	RB4	RB5	RB8
Pestalotiopsis disseminata	2.5 ± 0.10*	2 ± 0.3*	2.5 ± 0.20*	NI	NI
Fomes lamoensis	0.8 ± 0.03*	1.6 ± 0.06*	1.3 ± 0.04*	NI	1.8 ± 0.06*
Fusarium moniliforme	0.6 ± 0.01*	0.7 ± 0.01*	0.6 ± 0.03*	0.7 ± 0.03*	NI
Aspergillus niger	1 ± 0.03*	NI	0.3 ± 0.01*	0.4 ± 0.04*	NI
Fusarium oxysporum	0.5 ± 0.03*	0.2 ± 0.02	0.3 ± 0.03*	0.1 ± 0.02	NI
Fusarium semitectum	0.1 ± 0.04	0.6 ± 0.04*	0.4 ± 0.02*	0.7 ± 0.05*	0.4 ± 0.01*
Fusarium solani	0.7 ± 0.03*	0.8 ± 0.06*	0.7 ± 0.04*	0.8 ± 0.04*	0.2 ± 0.02
Rhizoctonia solani	0.8 ± 0.04*	0.8 ± 0.06*	0.7 ± 0.02*	0.7 ± 0.05*	0.2 ± 0.01

NI, No inhibition.
*Values are statistically significant over control ($P < 0.05$). Control was inoculated in the same way, but without the rhizobacterium.

Table 2. Biochemical tests for identification of the strains[a]

Strain	Organism	Biochemical test									
		Growth on MacConkey agar	Indole test	Methyl red test	Voges Proskauer test	Citrate utilization	H_2S production	Gas production	Casein hydrolysis	Esculin hydrolysis	Gelatin hydrolysis
RB1	*Bacillus cereus* (MTCC 8297)	NG	–	–	–	–	–	–	+	+	+
RB4	*Pseudomonas rhodesiae* (MTCC 8299)	+ (NLF)	–	–	–	+	–	–	+	–	+
RB5	*P. rhodesiae* (MTCC 8300)	+ (NLF)	–	–	–	+	–	–	+	–	+
RB3	*Streptomyces luteireticulii* (MTCC 8298)	–	–	–	–	NG	NT	NT	+	+	+
RB8	*Chromobacterium violaceum* (MTCC 8071)	+	–	–	–	+	–	–	+	–	+

Strain	Organism	Biochemical test								
		Starch hydrolysis	Urea hydrolysis	Nitrate reduction	Catalase test	Oxidase test	Tween 20 hydrolysis	Tween 40 hydrolysis	Tween 80 hydrolysis	Fluorescence
RB1	*B. cereus* (MTCC 8297)	+	+	+	+	+	+	+	–	NT
RB4	*P. rhodesiae* (MTCC 8299)	–	–	–	+	+	+	–	–	+
RB5	*P. rhodesiae* (MTCC 8300)	–	–	–	+	+	+	–	–	+
RB3	*S. luteireticulii* (MTCC 8298)	–	NT	–	+	+	NG	NG	–	NT
RB8	*C. violaceum* (MTCC 8071)	–	–	+	+	–	–	+	NT	NT

Strain	Organism	Acid production from							
		Glucose	Fructose	Xylose	Arabinose	Salicin	Mannose	Mannitol	Inositol
RB1	*B. cereus* (MTCC 8297)	+	+	NT	–	+	+	–	NT
RB4	*P. rhodesiae* (MTCC 8299)	–	+	+	–	NT	+	–	+
RB5	*P. rhodesiae* (MTCC 8300)	–	–	+	–	NT	+	–	–
RB3	*S. luteireticulii* (MTCC 8298)	+	+	–	–	+	NT	NT	–
RB8	*C. violaceum* (MTCC 8071)	NT	+	–	–	NT	NT	NT	NT

NT, Not tested; NG, No growth; +, Positive; –, Negative; NLF, Non-lactose fermenters.
[a]Conducted at MTCC, IMTECH, Chandigarh.

There was an increase in CFU/g of the introduced rhizobacteria as well as aerobic bacteria from day 10 to 20 (Figure 1). It is important to note that the population of the introduced rhizobacteria remains almost unchanged from day 20 to 30. RB1 and RB4 have the highest population of introduction in all the days.

The results of identification by IMTECH, Chandigarh are as follows: RB1 (MTCC 8297) *Bacillus cereus*, RB4 (MTCC 8299) *Pseudomonas rhodesiae*, RB5 (MTCC 8300) *Pseudomonas rhodesiae*, RB3 (MTCC 8298) *Streptomyces luteireticulii* and RB8 (MTCC 8071) *Chromobacterium violaceum* (Table 2). Table 2 shows the

Table 3. Effect of different consortia of PGPR strains on mature leaves of som plants[a]

Strain	Total chlorophyll (mg/g fr wt)	Carbohydrate (mg/g fr wt)	Free amino acid (mg/g fr wt)	Reducing sugar (mg/g fr wt)	Protein (mg/g fr wt)	Dry matter (mg/g fr wt)
1	1.109 ± 0.03	48.75 ± 0.70	1.35 ± 0.03*	0.15 ± 0.05*	3.95 ± 0.10*	0.873 ± 0.03
2	1.169 ± 0.06*	52 ± 0.90*	1.645 ± 0.04*	0.163 ± 0.06*	2.77 ± 0.02	0.88 ± 0.04*
3	1.362 ± 0.04*	58.75 ± 1.70*	1.845 ± 0.06*	0.168 ± 0.04*	4.225 ± 0.20*	0.827 ± 0.01*
C	1.052 ± 0.04	47 ± 1.80	0.88 ± 0.01	0.084 ± 0.03	3.525 ± 0.11	0.795 ± 0.02

[a]Values are mean of three replicates of 30 plants each ± SE.
*Values are statistically significant over the control ($P < 0.05$).
1, RB1 + RB3 + RB5; 2, RB4 + RB8; 3, RB1 + RB3 + RB4 + RB5 + RB8, C, Control.

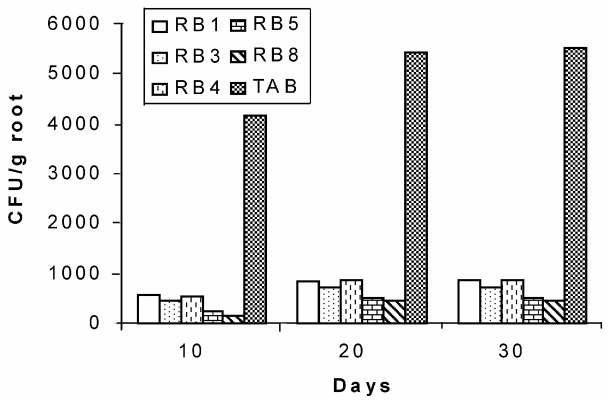

Figure 1. Population of the introduced bacteria (RB1, RB3, RB4, RB5, RB8) and total aerobic bacteria (TAB) on the rhizoplane of som roots (CFU/g root).

biochemical tests that were performed for identification of the strains (RB1, RB4, RB5, RB3, RB8) by IMTECH, Chandigarh.

In the NEIST campus, the growth responses of the potted som plants were variable and dependent on the rhizobacterial strain and the growth parameters (Figure 2 *a*, and *b*). The pot experiment showed the potency of five rhizobacterial strains as PGPR. All the PGPR strains provided a greater stimulation of bud emergence. The effect of the strains was prominent at 15 and 30 days after the plants had been treated with the strains, but the effect decreased after one month of treatment. Similar results were also obtained by Pan *et al.*[27]. Carbohydrate content, reducing sugars, free amino acids, total protein and total chlorophyll content increased significantly due to inoculation with the strains. However, treatment with the combination (RB1 + RB3 + RB4 + RB5 + RB8) was the best compared to other treatments (Table 3).

As evident from the 10% SDS–PAGE analysis, no specific protein was detected in the leaves of treated som plants. Banding pattern in the gel shows that the protein fractions were more intensely stained in the tender leaves of the plants treated with the strain RB1 + RB3 + RB4 + RB5 + RB8 (Figure 3 *b*). The difference in the banding intensity may be the result of physiological and biochemical changes in the treated plants, which in turn showed an enhanced biomass content compared to the control[28].

Treatment with the same strain combination was selected for spraying on large scale in the experimental som plantation farm of CMERTI. The som plants were sprayed and the effect of the strains was studied in the tender leaves in terms of increase in biochemical parameters on the fourth, 12th and 30th day of spraying (Table 4). There was significant variation among the som plants. Some som plants were considered to be the best in terms of increased biomass content. These plants will be considered for marker development and can be used for vegetative propagation.

Trehalase activity was found to be highest on day zero of the fifth instar for the silk gland, fat body and haemolymph, and decreased significantly at later stages (Figure 4 *a*). Except in haemolymph, activity in the tissues was high compared to the control. Insect eggs store carbohydrate in the form of glycogen and this reserve is trehalose from haemolymph that accumulates during the later stage of the fifth instar[29]. Since trehalase is involved in carbohydrate metabolism, it increases in the adult stage, i.e. fourth instar to pre-fifth instar. This increase in trehalase activity suggests that there is a definite need for its activity in the spinning process of silk glands[30].

There was no similarity in phosphorylase activity with trehalase, as the activity of the former tends to decrease in the last days of feeding of the fifth instar. This observation may be due to the fact that the insect stops eating at this stage and the undegraded glycogen is stored for energy during molting[31]. There were significant change in the larvae fed on treated som when compared with the larvae that did not feed on PGPR-sprayed som leaves. Phosphorylase activity was found to be the highest on the fifth day for silk gland, while in fat body and haemolymph the activity was found to gradually decrease in the later stage of the fifth instar (Figure 4 *b*). Increase in activity was observed to be comparable to the control.

Studies on pyruvate content in the larvae show that the level of pyruvate increases in the first stage of the fifth instar and declines later before spinning[31]. Silk gland, the site of silk synthesis, has high transaminase activity[32]. However, the 10% SDS–PAGE of the silk gland did not

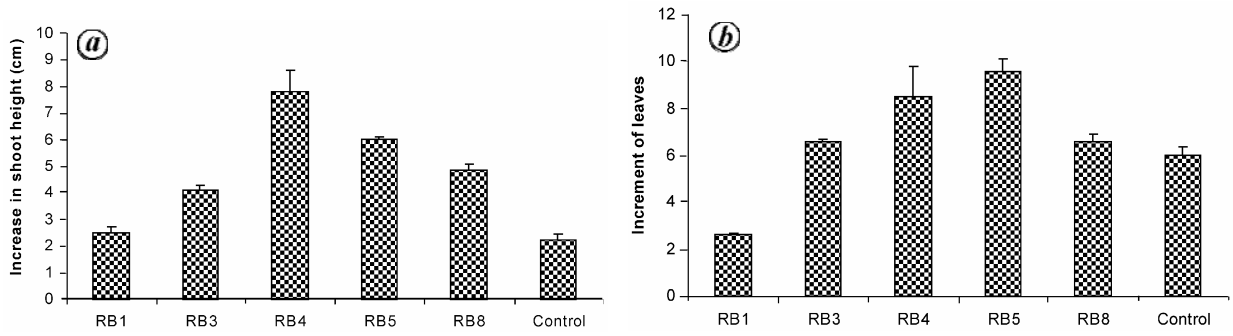

Figure 2. Increase in shoot height (cm) (***a***) and increment of leaves (***b***) of som potted plant treated with five rhizobacterial strains (RB1, RB3, RB4, RB5, RB8) and control.

Table 4. Effect of strain RB1 + RB3 + RB4 + RB5 + RB8 in young, tender som leaves 4, 12 and 30 days of spraying[a]

Treatment	Protein (mg/g fr wt)			Reducing sugars (mg/g fr wt)			Free amino acids (mg/g fr wt)		
Days of spraying	4	12	30	4	12	30	4	12	30
Control	0.015 ± 0.04	0.018 ± 0.02	0.102 ± 0.03	0.015 ± 0.02	0.018 ± 0.06	0.102 ± 0.02	0.2 ± 0.02	0.28 ± 0.04	1.75 ± 0.03
RB1 + RB3 + RB4 + RB5 + RB8	0.049 ± 0.03*	0.042 ± 0.06*	0.236 ± 0.04*	0.049 ± 0.01*	0.042 ± 0.04*	0.236 ± 0.04*	0.27 ± 0.02	0.45 ± 0.01*	2.075 ± 0.02*

[a]Values are mean of three replicates of 30 plants each ± SE.
*Values are statistically significant over the control ($P < 0.05$).

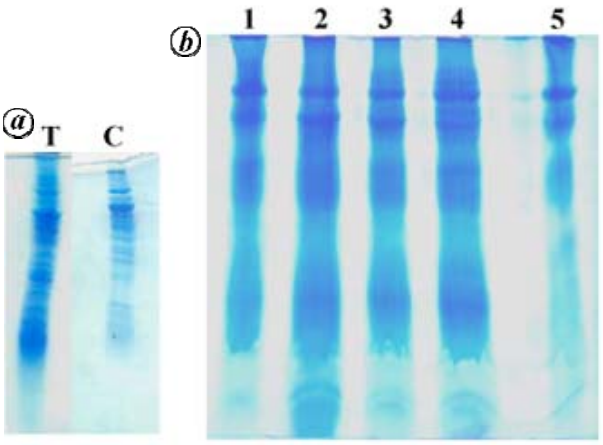

Figure 3. ***a***, 10% SDS–PAGE of silk gland from fifth instar larva. T, Treated; C, Control. ***b***, 12% SDS–PAGE of som leaves of PGPR-treated and untreated (control). Lane 1, Treatment RB1 + RB3 + RB5; lanes 2 and 3, Treatment RB2 + RB3 + B4 + RB5 + RB8; lane 4, Treatment RB2 + RB4 + RB8 and lane 5, Control.

Table 5. Effect of PGPR on cocoon parameters of *Antheraea assama*[a]

Treatment	Cocoon weight (g)	Shell weight (g)	Fibre content (g)
PGPR-treated	5.45 ± 0.4*	0.53 ± 0.2*	0.52 ± 0.3*
Control	4.87 ± 0.5	0.42 ± 0.1	0.37 ± 0.2

[a]Values are mean of three replicates of ten cocoons each ± SE.
*Values are statistically significant over the control ($P < 0.05$).
PGPR: RB1, RB3, RB4, RB5, RB8.

Table 6. Effect of PGPR on fibre quality of *A. assama*[a]

Treatment	Filament length (m)	Non-breakable filament length (m)
PGPR-treated	309 ± 10.4*	225 ± 4.9*
Control	252 ± 8.6	108 ± 1.3

[a]Values are mean of three replicates of ten cocoons each ± SE.
*Values are statistically significant over the control ($P < 0.05$).
PGPR: RB1, RB3, RB4, RB5, RB8.

show any induced protein band and was similar to that of the control larvae (Figure 3 *a*). In the present study, alanine amino transferase activity increases after feeding, but in the later stages there is a decline in the activity in both treated and control larvae. However, the activity (μg pyruvate released in 30 min/mg protein) was significantly high in the tissues of the larvae fed with the treated som leaves (Figure 4 *c*).

PGPR application has induced plant defence enzyme (such as phenylamine ammonia-lyase, peroxidase and polyphenoloxidase) activities in the leaf and root of betelvine, *Piper betle* L.[33]. The enhanced activities of the enzymes through the application of PGPR are reported in this study with tissues of muga silkworm *A. assama.*

The shell weight of the cocoons formed from the larvae fed with treated som leaves was higher than that of the

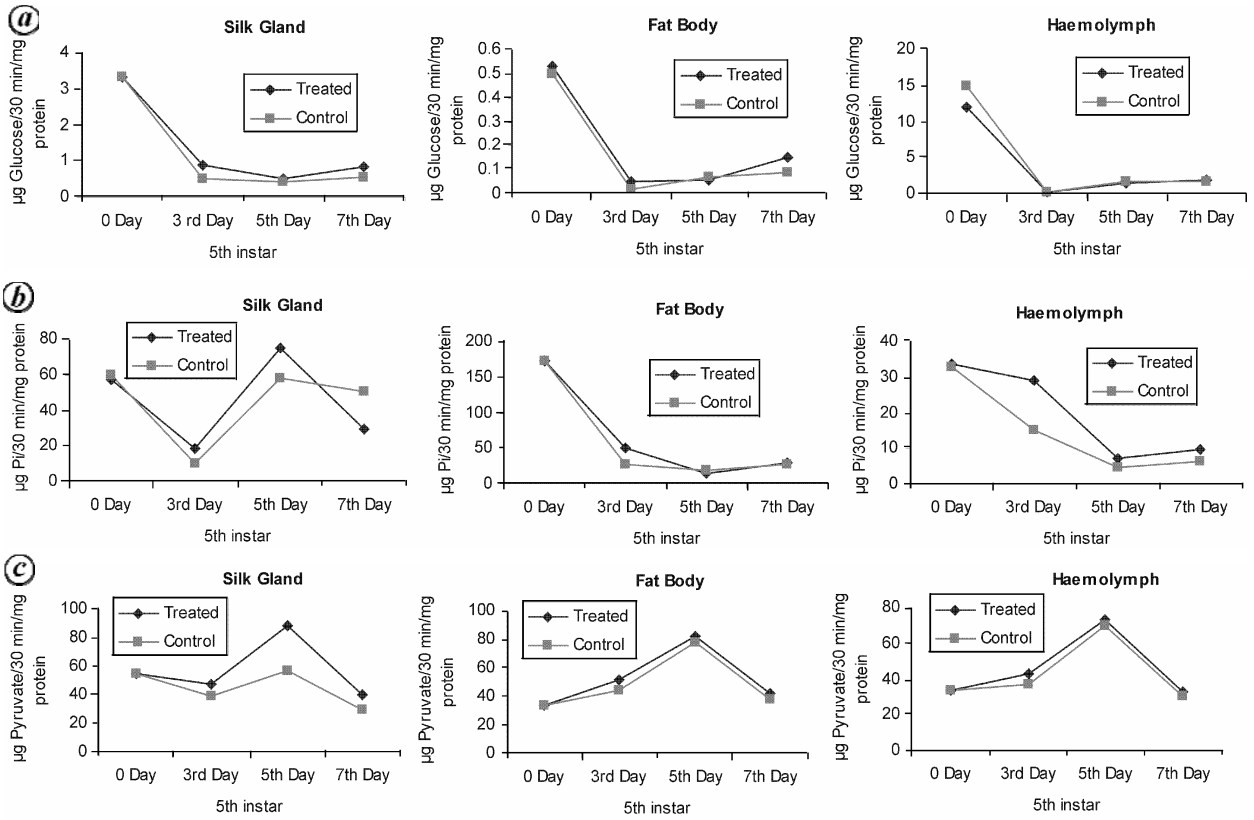

Figure 4. Trehalase (***a***), phosphorylase (***b***) and transaminase (***c***) activities in tissues of muga silkworm fed with som leaves treated with PGPR (RB1, RB3, RB4, RB5, RB8) and control.

control (Table 5). The cocoons were used for fibre estimation and there was considerable increase in fibre content. Moreover, the fibres were longer and have higher non-breakable filament length compared to the control (Table 6).

Based upon the growth-promoting activity in som plants using three combinations of strains, it can be concluded that PGPR (*Bacillus* spp., *Streptomyces* spp., *Pseudomonas* spp. and *Chromobacterium* spp.) caused maximum increase in growth of som plants, and fibre quantity and quality in muga silkworms. PGPR has also been reported to enhance the nutrient accumulation and growth of oil palm seedlings[34] and production of high-yielding and good quality banana[35]. There is no evidence of interaction of PGPR with the enzyme activity of larval tissues in silkworms. Hence, this study suggests that rhizobacteria could be an effective tool for enhanced biomass production in som plants, which in turn has an impact on the growth of silkworms to produce more silk fibre of good quality. Considering the above facts, the present investigation may prove useful to the sericulture farmers of Northeast India.

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Perfusion of antifungal agents through biofilms of *Candida* sp.

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An *in vitro* bioassay system for evaluation of perfusion of antifungal agents through biofilms of *Candida* species has been developed. Cellulose membrane filter was used as substrate material for biofilm development. The chosen drugs were not able to penetrate the biofilm even at high concentration (2.0 mg/ml). However, partial penetration was exhibited by members of the azole family. Scanning electron micrograph reveals encapsulation of the matrix throughout the biofilm, which restricts antifungal penetration. Of the two *Candida* species, *C. tropicalis* was less responsive to antifungal agents than *C. albicans*.

Keywords: Antifungal agents, biofilms, drug resistance, perfusion.

CANDIDA species are fungal pathogens, causing superficial or systemic infection in immunocompromised individuals[1–5]. They are ranked as fourth in the cause of nosocomial infections, third in catheter-related infections, second highest in colonization to infection rate and highest overall in crude mortality[4,6–9]. In general, candidal infections involve biofilm formation on the surface of implant devices such as catheters, shunts, stents, lenses, etc. and serve as a source of infection till the implant is removed[1,2,7,10]. This makes the *Candida* species a high-risk pathogen rather than still being considered as an opportunistic pathogen. Nevertheless, non-device related biofilm infections such as vaginitis and periodontitis are obstinate to treatment[6].

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