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Screening of Rhizobacterium, *Burkholderia* for Biocontrol of Bacterial Pathogens of Tropical Tasar Silkworm, *Antheraea Mylitta* D. And Induction of Growth in Silkworm Host Plant, *Terminalia Arjuna*

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Abstract: Plant growth promoting rhizobacterium, *Burkholderia* species was isolated from the soil of tropical tasar silkworm rearing plots by using serial dilution method. The isolated bacterium was characterized by using different biochemical test. *Burkholderia* species was tested against bacterial pathogens of the tropical tasar silkworm, *Antheraea mylitta*. The seedlings of tasar host plant, *Terminalia arjuna* treated with *Burkholderia* species during nursery plantation showed enhanced shoot and root length in comparison with untreated seedlings. Thus, *Burkholderia* species can be used for the induction and enhancement of growth in *T. arjuna* and also for the control of bacterial diseases of tropical tasar silkworm.

Key Words: PGPR, *Burkholderia*, Antimicrobial activity, Root induction, Shoot induction.

INTRODUCTION

Tropical Tasar silkworm (*Antheraea mylitta* Drury) is a polyphagous insect which feed on a number of food plants. However, it has food preference feeding primarily on *Terminalia tomentosa*, *Terminalia arjuna* and *Shorea robusta* and secondarily on more than two dozens of food plant. The host plants, which silkworm normally prefers are known as primary host plants. Other host plants,

where the silkworm can sustain its life, but normally do not prefer, are known as secondary host plants[1].

Due to quick deforestation of the silkworm host plants in tasar growing areas due to human interventions, the availability of food to tropical tasar silkworm is becoming little difficult. So there is an urgent need to replant the silkworm host plants in affected area. Since *Terminalia arjuna* is perennial plants, the growth of its seedlings in nursery takes more time. Hence, there is a need for identification of suitable growth promoting microorganisms which enhance the root and shooting in the stem cuttings of *Terminalia arjuna*.

A group of rhizobacteria that exert the beneficial effects on plant growth is referred as Plant Growth Promoting Rhizobacteria (PGPR). They belong to different genera and their endospores are tolerant to heat and desiccation. Large population of PGPR established on planting material presumably prevents or reduces the establishment of pathogens. Treatment of PGPR which were isolated from the rhizosphere of Muga silkworm host plant, Som (*Persea bombycina)* showed increase in chlorophyll content, free amino acid, total protein, reducing sugar, carbohydrate and dry weight and good cocoon yield, along with higher activity of the enzymes *viz*., trehalase, transaminase and phosphorylase in the silk gland, haemolymph and fat body[2].

Tropical Tasar silkworm is incited by different kinds of pathogens *viz*., microsporidia, bacteria, fungi and viruses. These pathogens cause considerable yield loss to an extent[3] of 40%. Among all the diseases, bacterial diseases are most prevalent during rearing season causing considerable yield loss. Three types of bacteria symptoms can be noticed in tropical tasar silkworm *viz*., Sealing of Anal Lips, Rectal Protrusion and Chain Type Excreta caused by different bacterial pathogens.

Some work has been done to isolate the phylloplane bacteria to treat against bacterial diseases of tropical tasar silkworm[4]. However, the information about the exploitation of rhizobacteria of host plants against bacterial diseases of tropical tasar silkworm is not available. Hence, the present work aims to isolate the rhizobacteria from the plots of tasar silkworm host plants, *Terminalia arjuna* and *Terminalia tomentosa* and study the efficacy of different isolated rhizobacterial isolates against the pathogenic bacterial isolates of tropical tasar silkworm and for induction of growth in silkworm host plants.

MATERIALS AND METHODS

Collection of soil: Dry soil sample were collected from the Arjun (*Terminalia arjuna*) and Asan (*Terminalia tomentosa*) plantation plot of Central Tasar Research and Training Institute Ranchi. Further soil sample were dried in room condition.

Serial dilution method and isolation of rhizobacterial species: The different concentration of soil solution was prepared by using standard protocol of serial dilution. The soil suspension (10^{-4}) of both Arjun and Asan plots were used to spread on Nutrient Agar (NA) plate along with other previous dilutions. After 24 hours of spreading, the plates were observed for the growth of bacterial colonies. The individual bacterial colonies were isolated and cultured on NA slants. The isolated bacteria were sub-cultured again on the NA slants and used for the screening against pathogenic bacterial species.

Mass screening of rhizobacterial species against pathogenic bacterial species (*In vitro* Inhibition Assay): Each pathogenic and antagonistic bacterial suspension was prepared in sterilized water and their initial concentrations were adjusted to approximately 10^8 colony-forming units (CFU)/ml. Each rhizobacterium was screened against all bacterial pathogens by using gel diffusion method. The promising bacterium was used for further studies.

Biochemical Characterization of promising rhizobacteria: The promising rhizobacteria was identified by using Gram staining reaction, KOH solubility test, Starch hydrolysis, Catalase test, Lipase activity and Casein hydrolysis.

Evaluation of bacteria for Plant Growth Promoting (PGP) parameters : Different tests such as root colonization, Indol Acetic Acid (IAA) production, Hydrogen Cyanide production, phosphate solubilization, Salicylic Acid production, volatiles production and antagonism were conducted to analyze the plant growth promoting (PGP) characters of *Burkholderia* spp.

Evaluation of physio-biochemical parameters of seedlings raised from *Burkholderia* treated stem cuttings: Leaf moisture content (MC) was determined on fresh weight basis using the following relating. To determine oven-dry weight, leaves were placed in an oven at 70°C for more than 24 h till the constant weight was obtained.

MC (%) = [100 × (Fresh weight – Oven-dry weight)/Fresh weight)]

The net photosynthesis rate was evaluated using universally accepted CI-340 Photosynthesis System. The biochemical characters of treated and control leaves such as chlorophyll estimation[5], total soluble protein content[6], total carbohydrate content[7] and crude fiber content[8] were estimated.

RESULTS

Isolation of bacterial species by serial dilution method: The bacterial colonies were noticed on the Nutrient Agar (NA) media in all the dilutions. The more number of single bacterial colonies were noticed in 10^{-4} dilution. The individual colonies were picked by using the inoculation loop and inoculated to NA slant for further experimentation.

Inhibition of pathogenic bacteria by antagonistic rhizobacteria (*In vitro* inhibition assay) : Among all the rhizobacterial isolates, the isolate 106 (*Burkholderia* sp.) showed antagonism against all the three different kinds of pathogenic bacteria while other isolates showed inhibition against one or two pathogenic bacteria whereas isolate 101 showed no reaction with pathogens. *Burkholderia* exhibited the zone of inhibition of pathogenic bacteria on par with the standard (Ampicillin) **(Table 1 & Figure 1).**

Table 1: Inhibition of different pathogenic bacteria by Rhizobacterial isolates

Bacteria isolated	*Micrococcus* sp. (Rectal Protrusion)	*Microbacterium* sp. (Sealing of Anal Lips)	*Serratia* sp. (Chain Type Excreta)
101	0	0	0
102	0	0	1.10 ± 0.27^{c}
103	0.30 ± 0.67^{d}	0	0.40 ± 0.25^{f}
106 (*Burkholderia* sp.)	1.09 ± 0.47^{a}	1.20 ± 0.45^{b}	1.50 ± 0.34^{b}
107	0	0	0.72 ± 0.45^{d}
108	1.00 ± 0.29^{c}	0	0.25 ± 0.37^{g}
109	0	0.80 ± 0.35^{c}	0
Standard	1.74 ± 0.75^{b}	1.74 ± 0.25^{a}	1.72 ± 0.25^{a}

The values represent the mean of three replications with standard error, values with different small alphabets are significant different according to Duncan's Multiple Range Test (DMRT, ($P<0.05$).

Fig. 1: Representative Photographs showing antagonism of *Barkholderia* species against different pathogenic bacterial species.

Biochemical characterization of promising rhizobacteria: The antagonistic bacteria showed negative reaction to gram staining, starch hydrolysis, Hydrogen sulphide production and fluorescence test. The bacterium showed positive reaction to catalase activity, lipase activity, casein hydrolysis and KOH solubility test. The results confirm that, the promising antagonistic bacterium is *Burkholderia* species **(Table 2).**

Table 2: Biochemical Characterization of promising PGPR (106)

Tests conducted	**Results**
Gram Staining	-ve
Starch Hydrolysis	-ve
Catalase activity	+ve
Lipase activity	+ve
Casein hydrolysis	+ve
H2S production	-ve
Fluorescence	-ve
KOH solubility test	+ve

Evaluation of bacteria for Plant Growth Promoting (PGP) parameters : The evaluation of *Burkholderia* bacterium for PGP parameters showed positive results to root colonization, Indol Acetic Acid (IAA) production, Hydrogen Cyanide production and antagonism, whereas negative results were observed in phosphate solubilization, Salicylic Acid production and volatiles production tests **(Table 3).**

Table 3: Plant Growth promoting characters of *Burkholderia* sp.

Tests conducted	Results
Root Colonization	+ve
IAA production	+ve
Hydrogen Cyanide production	+ve
Antagonism	+ve
Phosphate Solubilization	-ve
Salicylic acid production	-ve
Volatiles production	-ve

Effect of *Burkholderia* treatment on the shoot length and root length of *Terminalia arjuna* cuttings: Enhancement in the length of root and shoot were noticed in the cuttings with *Burkholderia* treatment in comparison with control ones. Almost two fold increase in shoot and root length was noticed in bacterium treated cutting in comparison with control ones **(Figure 2 & 3).**

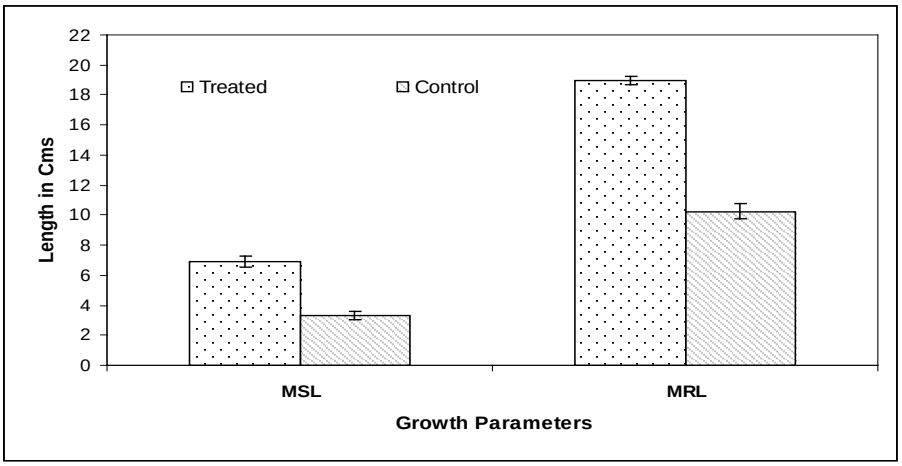

Fig. 2: Comparative evaluation of growth parameters in *T. arjuna* cuttings.

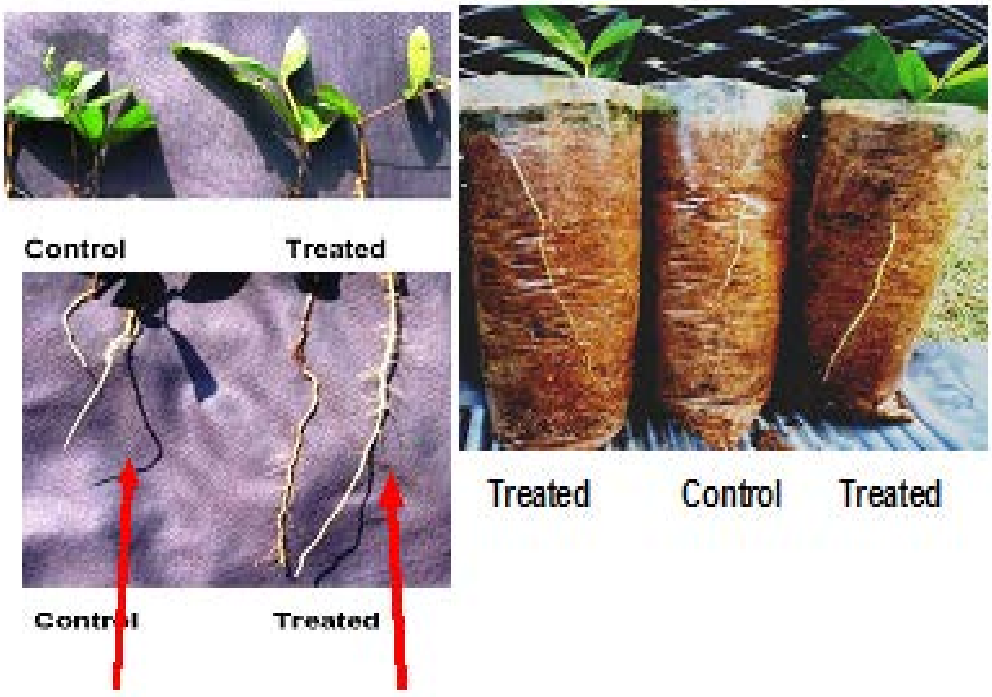

Fig. 3: Effect of *Burkholderia* suspension treatment on the Root and shoot induction in *Terminalia arjuna* cuttings.

Evaluation of physio-biochemical parameters of seedlings raised from *Burkholderia* treated cuttings : The physiological parameters such as chlorophyll estimation, total soluble protein content, total carbohydrate content, leaf moisture content (MC), net photosynthesis rate showed the increasing trend, whereas crude fiber content showed declined concentration in treated plants in comparison with control cuttings **(Figure 4)**.

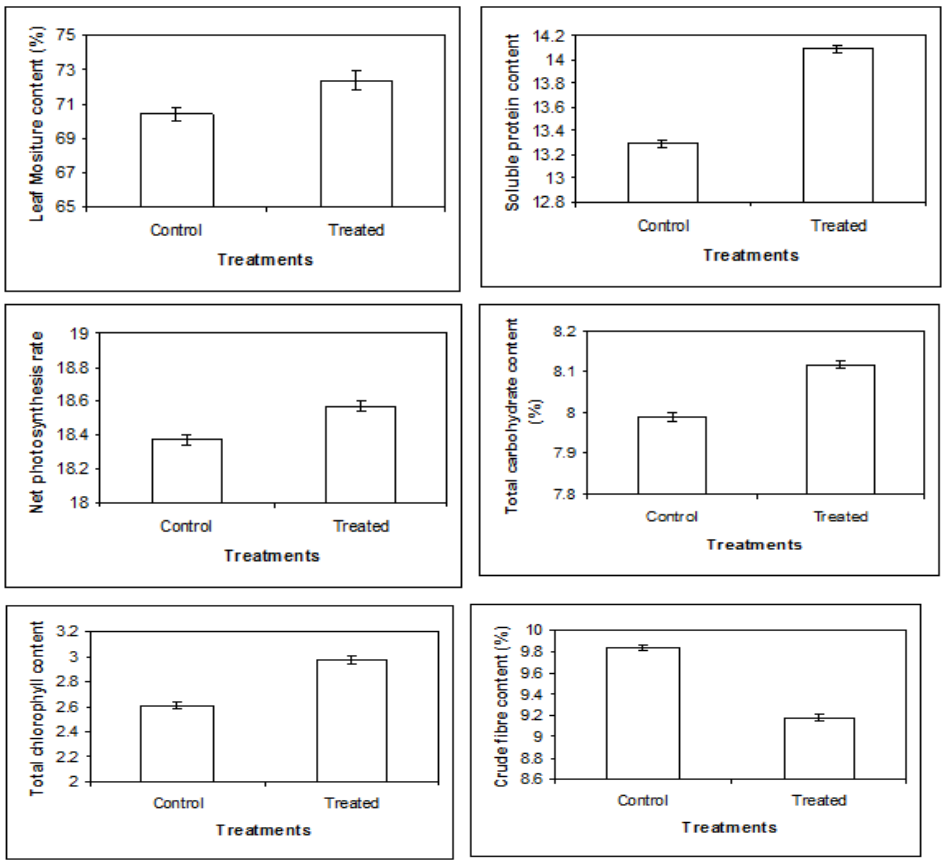

Fig. 4: Effect of *Burkholderia* suspension treatment on the physiological and Biochemical parameters of *Terminalia arjuna* cuttings.

DISCUSSION

Tropical tasar silkworm, *Antheraea mylitta* Drury (Order: Lepidoptera, Family: Saturniidae) is a semi-domesticated sericigenous insect which is distributed all over India in different ecological pockets. It is exploited commercially for the production of tasar silk. Tasar silkworm is a polyphagous insect feeding primarily on *Terminalia arjuna, Terminalia tomentosa* and *Shorea robusta* and secondarily on more than two dozens of food plants. Tasar silkworm host plant, *Terminalia arjuna* come under the "very difficult to root" group of plants. Efforts on clonal propagation through cuttings are being constantly made and recently, some success was made to regenerate it through softwood (Juvenile) and semi hardwood (Leaf Node) cuttings[9]. A deep and proliferated root is an effective means of avoiding drought. Drought tolerant accessions of a plant species should have deep root system. An increase in water deficiency causes increase in root: shoot ratio. Drought induced preferential root growth may possibly constitute an adaptive mechanism[10].

The results of present study showed the enhanced growth of shoot and roots, along with increase in necessary physio-biochemical parameters in saplings raised from stem cuttings treated with *Burkholderia* sp. which thus can help in enhanced leaf production resulting in increase in cocoon production. The present findings are in conformity with earlier study[2]. Plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of Som plants (*Machilus bombycina*) when tested in combinations showed increase in chlorophyll content, free amino acid, total protein, reducing sugar, carbohydrate and dry weight. Five strains showing growth promoting activity were selected and all the combinations had positive effect on the biochemical parameters studied, but the different combinations of strains produced the best result. 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[2].

Since bacterial pathogens are contributing for the considerable decrease in tropical tasar cocoon production, there is urgent need for the identification of biocontrol agent which controls all the three types of bacteria. The present study also confirms that rhizobacteria (*Burkholoderia* spp.) showed the antagonism on the all three bacterial pathogens infecting tropical tasar silkworm. Similar work has been done by Xunli *et al.*[11], who observed that the rhizobacteria isolated from the soil of Tai Mountain showed antagonism against various pathogenic bacteria and fungus of silkworm.

CONCLUSION

From the present experimental study, it can be concluded that the isolated rhizobacteria (*Burkholderia* species) can be used as potent antibacterial biocontrol agent. Similarly, the bacterium can also be exploited for the induction of roots and shoots in the *Terminalia* species nursery.

REFERENCES

1. M.Deka and M.Kumari, Comparative Study of the Effect of Different Food Plant Species on Cocoon Crop Performance of Tropical Tasar Silkworm (Antheraea mylitta Drury). International Journal of Research in Chemistry and Environment, 2013, 3(1):99-104.
2. B.G.Unni,U. Bora, H.R. Singh, D. Kumar, and B.Devi, High yield and quality silk fiber production by muga silkworm, Anthereae assama through application of plant growth promoting rhizobacteria. *Current Science*, 2008, 94: 768-774.
3. D.N.Sahay, D. K. Roy and A.Sahay, Diseases of tropical tasar silkworm, *Antheraea mylitta* D., Symptoms and control measures, In: *Lessons on Tropical Tasa*r. Ed. By K. Thangavelu, Central Tasar Research and training Institute, Piska Nagri, Ranchi, 2000, 104.
4. D.K.Roy, A. Sahay, and D.N.Sahay, Leaf Surface Microflora for Tasar Crop Improvement. Indian Silk,2006, 45 (1-12): 19-21
5. J.N. Anderson and N.K. Boardman. Studies on greening of dark brown bean plants. VI. Development of phytochemical activity. *Aust. J. Bio. Sci.*, 1964, **17**, 93-10
6. O.H. Lowry, J.H. Rosebrough, A.L. Farr and all R.J. Protein measurement with Folin-Phenol reagent. *J. Bio. Chem.*, 1951, **193**, 265-275.
7. J.E. Hedge and B.T. Hofreiter. Carbohydrate chemistry. 17 (eds), Whistler R. L. and Be Miller J. N. Academic Press, 1962, New York.
8. A.J. Maynard. Methods in food analysis. Academic Press, New York. P, 1970, 176.
9. U.P.S. Sinha, C.M. Bajepeyi, A.K.Sinha, B.N.B. Chari, and B.R.R.P.Sinha, Food consumption and utilization in *Antheraea mylitta* Drury larvae. *Int. J. Wild Silkmoth and Silk*., **5**, 182-186 **(2000)**
10. Z.Zlatev and F.C.Lidon, An overview on drought induced changes in plant growth, water relations and photosynthesis. *Emir. J. Food Agric*. 2012. 24 (1): 57-72.
11. L.Xunli,S. Changpo,M.A. Yingfei, W. Chao, and L.Kaiqi, Isolation and Identification of An Antagonistic Bacteria Strain Against Silkworm Pathogens. Acta Sericologica Sinica. 2004,03.

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