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## RESEARCH PUBLICATIONS

(RG SCORE- 9.87, h-index-7, RG citation- 271; Google Scholar citation- 357, h- index-10)

<u>Published In National and International Journals</u> <u>International Journals= 11</u> National Journals= 12

 Evaluation of *Ochrobactrum anthropi* TRS-2 and its talc based formulation for enhancement of growth of tea plants and management of brown root rot disease.
 U. Chakraborty, B.N.Chakraborty, M. Basnet and A.P.Chakraborty
 *Journal of Applied Microbiology*, 107: 625-634 (2009)
 NAAS rating 2019 – 8.16 (Jrn ID-J069)
 Thomson Reuters (SCI) impact factor -2.386

2. Influence of *Serratia marcescens* TRS-1 on growth promotion and induction of resistance in *Camellia sinensis* aginst *Fomes lamaoensis*U. Chakraborty, B.N.Chakraborty and A.P.Chakraborty *Journal of Plant Interactions*, 5(4): 261-271 (2010)
NAAS rating 2019 – 7.66 (Jrn ID J422)
Thomson Reuters (SCI) impact factor- 1.628

**3.** Protection of tea *plants* against pest and pathogen through combined application of pesticide and plant growth promoting rhizobacterium

A.P.Chakraborty, B.N.Chakraborty and U. Chakraborty Journal of Mycology and Plant Pathology, 40(4): 519-531 (2010) NAAS rating 2019–5.79 (Jrn ID- J363)

**4.** Plant growth promotion and amelioration of salinity stress in crop plants by a salt-tolerant bacterium

Usha Chakraborty, Swarnendu Roy, **Arka Pratim Chakraborty**, Pannalal Dey and Bishwanath Chakraborty

Recent Research in Science and Technology, 3(11): 61-70 (2011)

5. Dual application of *Bacillus pumilus* and *Glomus mosseae* for improvement of health status of mandarin plants
U. Chakraborty, B.N.Chakraborty, S.Allay, U.De and A.P.Chakraborty

Acta Horticulturae, 892: 215-229 (2011)

6. Evaluation of *Bacillus megaterium* and *Serratia marcescens* and their bioformulations for promoting growth of *Camellia sinensis* Chakraborty U, Chakraborty BN and **Chakraborty AP** *International Journal of Tea Science*, 8(1): 69-80 (2012) NAAS rating 2016 – 2.63 (Jrn ID- I179)

7. Induction of plant growth promotion in *Camellia sinensis* by *Bacillus megaterium* and its bioformulations

U. Chakraborty, B.N.chakraborty and **A.P.Chakraborty** *World Journal of Agricultural Sciences*, 8(1): 104-112 (2012)

8. Biological control sclerotial blight of tea using Arbuscular Mycorrhizal fungus and plant growth promoting rhizobacterium
B.N.Chakraborty, U. Chakraborty, U.De and A.P.Chakraborty *International Journal of Tea Science*, 8(4): 27-35 (2012)
NAAS rating 2016 – 2.63 (Jrn ID- I179)

9. Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria
U.Chakraborty, B.N.Chakraborty, A.P.Chakraborty and P.L.Dey *World Journal of Microbiology and Biotechnology*, 29: 789-803 (2013)
NAAS rating 2019 – 8.10 (Jrn ID W024)
Thomson Reuters (SCI) impact factor- 2.100

10. Plant growth promoting Rhizobacteria mediated improvement of health status of tea plants
U. Chakraborty, B.N.Chakraborty, A.P.Chakraborty, K.Sunar and P.L.Dey *Indian Journal of Biotechnology*, 12: 20-31 (2013)
NAAS rating 2019 – 6.37 (Jrn ID I043)
JCR impact factor (2017)- 0.368

**11.** Water and salt stress alleviation in wheat induced by rhizosphere bacteria with multi-functional traits.

Chakraborty U, Chakraborty BN, Chakraborty AP and Dey PL.

International Journal of Bioresource and Stress Management, 4(2): 214-219 (2013). NAAS rating 2019 – 4.65 (Jrn ID 1179)

12. Molecular identification and immunological characterization of plant growth promoting rhizobacteria of *Camellia sinensis*B.N. Chakraborty, U. Chakraborty, A.P. Chakraborty and P.L. Dey *International Journal of Tea Science*, 10(3&4): 41-52 (2014)
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13. Bacillus megaterium from tea rhizosphere promotes growth and induces systemic resistance in tea against *Sclerotium rolfsii*A.P. CHAKRABORTY, B.N. CHAKRABORTY and U. CHAKRABORTY *Indian Phytopathology*, 68(3) (2015)
NAAS Rating 2019 -5.90 (Jr, ID-1104)

**14.** Induction of resistance in *Camellia sinensis* against *Sclerotium rolfsii* by dual application of *Rhizophagus fasciculatus* and *Bacillus pumilus* Bishwanath Chakraborty, Usha Chakraborty, Utanka Kumar De and **Arka Pratim** 

Bishwanath Chakraborty, Usha Chakraborty, Utanka Kumar De and Arka Pratim Chakraborty

Archives of Phytopathology and Plant Protection, 48: 825-840, 2016. SJR Impact Factor: 0.209; SNIP Impact Factor: 0.345. (Scopus).

**15.** Serological and molecular detection of *Bipolaris sorokiniana* Sacc. causing Spot blotch disease of wheat

B. N. CHAKRABORTY, U. CHAKRABORTY, **A. P. CHAKRABORTY** AND P. SASHANKAR

*Journal of Mycopathological Research*, 54(1): 117-125, 2016 (ISSN 0971-3719). NAAS rating 2019 – 4.90 (Jr. ID- J364)

**16.** Resistance in tea plants against root rot pathogens induced by arbuscular mycorrhizal fungi and plant growth promoters

U. CHAKRABORTY, B. N. CHAKRABORTY, A. P. CHAKRABORTY AND U.K.DE Journal of Mycopathological Research, 54(2) : 211-224, 2016 (ISSN 0971-3719). NAAS rating 2019 – 4.90 (Jr. ID- J364)

**17.** Development of Immunological Formats for Detection of *Bipolaris sorokiniana* in Wheat Leaf and Strategies for Induction of Resistance against Spot Blotch Disease using Bioinoculants

**A.P. Chakraborty**, U. Chakraborty and B.N. Chakraborty *Journal of Mycology and Plant Pathology*, 46(1): 21-37, 2016. NAAS rating 2019 – 5.79 (Jrn ID- J363) 18. Evaluation of Streptomyces and non- Streptomyces Actinomycetes isolates for growth promotion in *Vigna radiata* and their use as biocontrol agent against *Sclerotium rolfsii* Pushpanjali Ray, Arka Pratim Chakraborty and Bishwanath Chakraborty *NBU Journal of Plant Sciences* (ISSN NO-09746927)- 10(1): 73-79, 2016.

**19.** Biochemical and molecular characterization of *Streptomyces* species isolated from Agricultural field of North Bengal and evaluation for growth improvement and suppression of sclerotial blight diseases of *Vigna radiata* 

Pushpanjali Ray, **Arka Pratim Chakraborty** and Bishwanath Chakraborty *Journal of Mycology and Plant Pathology*, 46(4): 344-354, 2016. NAAS rating 2019 – 5.79 (Jrn ID- J363)

**20.** PGPR in managing root rot disease and enhancing growth in mandarin (*Citrus reticulata* Blanco.) seedlings

B.N. Chakraborty, S. Allay, A.P. Chakraborty and U. Chakraborty *Journal of Horticultural Sciences*, 11(2): 104-115, 2017
NAAS rating 2019 – 3.43 (Jrn ID- J273)

**21.** Biochemical Responses of *Sorghum bicolor* and *Triticum aestivum* to spot blotch disease and induction of resistance by Plant Growth Promoting Rhizobacteria . Priyanka Bhattacharjee, Jayanwita Sarkar, **Arka Pratim Chakraborty**, Usha Chakraborty and Bishwanath Chakraborty

*Journal of Mycology and Plant Pathology*, 47(3): 251-261, 2017. NAAS rating 2019 – 5.79 (Jrn ID- J363)

**22.** Bacillus safensis from wheat rhizosphere promotes growth and ameliorates salinity stress in wheat

U Chakraborty, B N Chakraborty, P L Dey and A P Chakraborty Indian Journal of Biotechnology vol 17, JULY 2018 NAAS rating 2019 – 6.37 (Jrn ID I043) JCR impact factor (2017)- 0.368

**23.** Biochemical responses of wheat plants primed with *Ochrobactrum pseudogrignonense* and subjected to salinity stress

U. chakraborty, B.N.Chakraborty, P.L.Dey, A.P.Chakraborty and J.Sarkar

Agricultural Research Journal (Springer)

https://doi.org/10.1007/s40003-018-0394-7 NAAS rating 2019- 5.60 (Jrn ID A090)

### ACCEPTED FOR PUBLICATION

 Analyses of soluble proteins and phenolics in susceptible and resistant wheat genotypes against *Bipolaris sorokiniana* causing spot blotch disease
 P. Chakraborty, U. Chakraborty and B. N. Chakraborty
 *Journal of Mycopathological Research*, 58(1&2) April 2020 (ISSN 0971-3719).
 NAAS rating 2019 – 4.90 (Jr. ID- J364)

### In Proceeding volume:

1. Chakraborty AP, Chakraborty BN and Chakraborty U. Application of bioformulations of PGPRs for plant growth promotion in tea seedlings. *Microbial Resources for Crop Improvement* (Eds. Bishwanath Chakraborty and Usha Chakraborty), Satish Publishing House, 101-110, (2013), ISBN-978-93-81226-39-1.

2. Chakraborty BN, Chakraborty U, De U, Chakraborty AP and Rai K. Dual application of *Glomus mosseae* and *Bacillus pumilus* in enhancing growth of tea and suppression of sclerotial blight disease. *Microbial Resources for Crop Improvement* (Eds. Bishwanath Chakraborty and Usha Chakraborty), Satish Publishing House, 69-83, (2013), ISBN- 978-93-81226-39-1.

3. Chakraborty U, Chakraborty BN, Roy S, Dey PL and **Chakraborty AP**. Isolation, biochemical and molecular characterization of salt-tolerant bacteria from rhizosphere of *Cynodon dactylon-* a facultative halophyte. *Microbial Resources for Crop Improvement* (Eds. Bishwanath Chakraborty and Usha Chakraborty), Satish Publishing House, 239-250, (2013), ISBN- 978-93-81226-39-1.

4. Growth enhancement of tea seedlings induced by application of *Serratia marcescens* and *Bacillus megaterium* and associated biochemical changes.

A.P. Chakraborty, U. Chakraborty and B.N. Chakraborty

In: Molecular and Biotechnological approach to resource utilization- Microbes to Angiosperms (Edited by Samit Ray and Sukanta K. Sen), Levant Books Publishing House, 127-142, (2015), ISBN-978-93-84106-04-1.

### <u>In Book Chapter:</u>

1. Growth Promoting Rhizobacteria: Diversity, Mechanisms of action and Perspectives in Agriculture.

U. Chakraborty, B.N. Chakraborty, **A.P. Chakraborty**, K. Sunar and P.L. Dey. In: *Review of Plant Pathology* (Eds. B.N.Chakraborty and U.Chakraborty) vol.6: 215-268, (2014), Scientific Publishers, Jodhpur, ISBN-978-81-7233-916-6, ISSN-0972-9712.

2. Role of Microorganisms in Alleviation of Abiotic Stresses for Sustainable Agriculture. Usha Chakraborty, Bishwanath Chakraborty, Pannalal Dey and Arka Pratim Chakraborty (2015) In: *Abiotic Stresses in Crop Plants* (Eds U. Chakraborty and B.N. Chakraborty) CAB International pp.232-254, ISBN- 978-1-78064-373-1.

3.Functional and Genetic Diversity of Bacterial Isolates from Soil and Utilization of their Beneficial Traits for Crop Improvement.

U. Chakraborty, B. N. Chakraborty, P. L. Dey, K. Sunar and A. P. Chakraborty (2016) *In: Biodiversity and Sustainability: Opportunities and Challenges* (Eds.: Rakhee Das Biswas and Abhijit Sarkar), 59-76, ISBN: 978-93-5254-104-1.

**Book Published for UG Students:** 

Model Objective Question Bank- Botany For B.Sc. Undergraduate Students Author: Arka Pratim Chakraborty Publishing house: LAP LAMBERT Academic Publishing; https://www.lap-publishing.com https://www.morebooks.shop Published on: 2020-04-16

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**Blurb/Short text:** The book is a simple collection of probable questions on different topics of Botany that occurred in the mind of the author. The book has been designed to give a preliminary idea about the probable questions as well as to cater to students of undergraduate and students appearing for various competitive examinations. The author tries to include questions from the different sectors of Botany- Taxonomy, Phycology, Biochemistry, Anatomy, Economic botany, Archegoniates, Cell Biology, Genetics, Embryology, and Plant Physiology.

Keywords: Botany, taxonomy, anatomy, Embryology, phycology, Biochemistry, Cell Biology, genetics, PLANT PHYSIOLOGY, Archegoniates



RESEARCH PAPERS ATTACHED IN SEQUENCE



### ORIGINAL ARTICLE

# Evaluation of *Ochrobactrum anthropi* TRS-2 and its talc based formulation for enhancement of growth of tea plants and management of brown root rot disease

U. Chakraborty, B.N. Chakraborty, M. Basnet and A.P. Chakraborty

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#### Keywords

disease control, induced resistance, plant growth promoting rhizobacteria, root rot, tea.

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#### Abstract

Aim: To evaluate *Ochrobactrum anthropi* TRS-2 isolated from tea rhizosphere and its talc based formulation for growth promotion and management of brown root rot disease of tea.

Methods and Results: Ochrobactrum anthropi TRS-2, isolated from tea rhizosphere could solubilize phosphate, produce siderophore and IAA in vitro and also exhibited antifungal activity against six test pathogens. Application of an aqueous suspension of O. anthropi to the rhizosphere of nursery grown tea seedlings of five varieties of tea (TV-18, T-17, HV-39, S-449, UP-3 and) led to enhanced growth of the treated plants, as evidenced by increase in height, in the number of shoots and number of leaves per shoot. Treatment with O. anthropi also decreased brown root rot of tea, caused by Phellinus noxius. Multifold increase in activities of chitinase,  $\beta$ -1,3-glucanase, peroxidase and phenylalanine ammonia lyase in tea plants was observed on application of O. anthropi to soil followed by inoculation with P. noxius. A concomitant increase in accumulation of phenolics was also obtained. Further, talc based formulation of O. anthropi was prepared and its survival determined every month up to a period of 12 months. Ochrobactrum anthropi could survive in the formulation up to a period of 9 months with a concentration of 7.0  $\log_{10}$  CFU g<sup>-1</sup>, after which there was a decline. Talc formulation was as effective as aqueous suspensions in both plant growth promotion and disease suppression.

**Conclusion:** Ochrobactrum anthropi, either in aqueous suspension or as talc formulation induced growth of tea plants and suppressed brown root rot disease. It induced defense responses in tea plants.

Significance and Impact of the Study: *Ochrobactrum anthropi* and its talc based formulation can be considered as an addition to available plant growth promoting rhizobacteria (PGPR) currently being used for field application. The present study offers a scope of utilizing this bacterium for growth promotion and disease management which would help in reduction of the use of chemicals in tea plantations.

### Introduction

The rhizosphere, a zone very rich in nutrients, supports large microbial populations, which exert beneficial, neutral or detrimental effects on plant growth. Plant growth promoting rhizobacteria, first defined by Kloepper and Schroth (1978), include those bacteria, which, on inoculation into the soil, colonize the roots of plants and enhance plant growth. With more and more emphasis on organic farming, efforts are on to isolate and identify beneficial microbes, and hence, plant growth promoting rhizobacteria (PGPRs) are finding increasing applications today as biofertilizers and bioprotectants (Vessey 2003). Undoubtedly, excess use of chemical fertilizers and

### **RRST-Biochemistry**



# Plant Growth Promotion and Amelioration of Salinity Stress in Crop Plants by a Salt-Tolerant Bacterium

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| Article Info  | Abstract   |
|---|--|
| Article History   | Out of a large number of bacteria isolated from the rhizosphere of <i>Cynodon dactylon</i> , a facultative belophyte rise were found to be highly got telerant growing in putrient error.  |
| Received : 27-06-2011<br>Revisea : 25-08-2011<br>Accepted : 04-09-2011                | medium supplemented with 10% NaCl. Morphological, biochemical, and molecular characterization of all the isolates was done. RAPD analysis to determine relatedness among the nine bacteria were carried out, which revealed that all nine isolates could be  |
| *Corresponding Author   | grouped into 2 main clusters. In order to determine whether the isolates possess plant   |
| Tel : +91-3532776337<br>Fax : +91-3532699001<br>Email:<br>chakrabortyusha@hotmail.com | growth promoting activities, <i>in vitro</i> tests were done initially. Based on the <i>in vitro</i> tests the isolate 'S4' was selected as a potential salt-tolerant PGPR. This bacterium was identified as <i>Bacillus cereus</i> . For <i>in vivo</i> tests, this bacterium was applied as an aqueous suspension to the rhizosphere of 3 plants- <i>Vigna radiata, Cicer arietinum and Oryza sativa;</i> a set of each plant was also applied with 200mM NaCl solution 3 times a week after 15 days of growth. Results revealed that the bacterium could promote growth of the seedlings significantly which showed increased height, number and length of leaves, as well as root and shoot biomass. The ability of the bacterium to solubilize soil phosphate was also confirmed. <i>B.cereus</i> could also elicit antioxidant responses against salt stress in the plants, as evidenced by increased activities of enzymes such as superoxide dismutase, peroxidase, ascorbate peroxidase and catalase. Besides, it could also induce the activities of defense enzymes such as chitinase, $\beta$ -1, 3 glucanse and phenyl alanine ammonia lyase associated with induced systemic resistance. Results imply that the bacterium acts through a |
|   | combination of direct and indirect mechanisms.   |
| ©ScholarJournals, SSR   | Key Words: Vigna radiata, Cicer arietinum, Oryza sativa, salt-stress, PGPR   |
|   |  |

### Introduction

Salinity is one of the most critical constraints which hamper agricultural productions in many areas worldwide. Globally, some 20% of irrigated land (450,000 km<sup>2</sup>) is saltaffected, with 2,500–5,000 km<sup>2</sup> of production lost every year as a result of salinity [1]. Therefore, with increasing population and rise in food demand, the utilization of salt-affected soils for agriculture will become necessary to feed the growing population of the world. For this, evolving low cost, easily adaptable technologies become essential and are now considered as a major challenge. Extensive research is going on worldwide to develop strategies for coping with abiotic stresses through development of tolerant varieties, shifting the crop calendars, production of transgenics etc [2].

One of the recently gaining practices of counteracting the adverse effects of salinity on plant growth includes the implementation of salt-tolerant bacteria with natural growth promoting ability in such conditions. Plant growth promoting rhizobacteria, first defined by Kloepper and Schroth [3] include those bacteria, which, on inoculation into the soil, colonize the roots of plants and enhance plant growth. The term 'induced systemic tolerance' (IST) has been proposed for PGPR-induced physical and chemical changes in plants that result in

enhanced tolerance to abiotic stress [4]. In comparison to reports of inducing systemic resistance in plants by PGPR, fewer reports have been published on PGPR as elicitors of tolerance to abiotic stresses, such as drought, salt and nutrient deficiency or excess [4].

Certain soil bacteria can help the plants to avoid or partially overcome a variety of environmental stresses. Yildirim et al. [5] reported the mitigation of salt stress in Raphanus sativus by plant growth promoting rhizobacteria like Staphylococcus kloosii and Kocuria erythromyxa. PGPRelicited induced systemic tolerance (IST) aid the growth of plants under abiotic stresses by producing various antioxidants, which result in the degradation of reactive oxygen species (ROS) [6]. Production of IAA or unknown determinants can increase root length, root surface area and the number of root tips, leading to enhanced uptake of nitrate and phosphorous [7, 8]. PGPRs have also been reported to protect plants from various pathogens by activating defense genes, for example those encoding chitinase,  $\beta$ -1, 3-glucanase (GLU), peroxidase (POX), phenylalanine ammonia lyase (PAL) etc. [9].

### Water and Salt Stress Alleviation in Wheat Induced by Rhizosphere Bacteria with Multi-functional Traits

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### Keywords

Wheat, PGPR, water stress, salinity, antioxidants

### Abstract

Twenty-seven (W1-W27), twenty-three (IP1-IP23) and nine (S<sub>1</sub>-S<sub>5</sub>, N<sub>1</sub>, N<sub>2</sub>, N<sub>3</sub> and N<sub>4</sub>) bacterial isolates from wheat (Triticum aestivum), blady grass (Imperata cylindrica) and Bermuda grass (Cynodon dactylon) rhizosphere which were found to be highly salttolerant were further tested for PGPR characteristics in vitro. Those bacterial isolates showing positive responses in vitro were identified by morphological, biochemical and 16SrDNA sequencing to be Ochrobactrum pseudogregnonense (IP8), Bacillus safensis (W10), Bacillus cereus (S.). In vivo studies on salinity using B.cereus (S.), N<sub>2</sub> and N<sub>2</sub>. revealed that all three isolates could promote growth of wheat plants significantly in terms of increased length of leaf as well as root and shoot biomass. The isolates could also elicit antioxidant responses against salt stress in wheat, as evidenced by increased activities of anti-oxidative enzymes at different hours of treatment. B. safensis and O. pseudogregnonense which were found to be drought tolerant could promote growth in six varieties of wheat tested in terms of increase in root and shoot biomass, height of plants, yield, as well as increase in chlorophyll content. Besides, the wheat plants could withstand water stress more efficiently in presence of the bacteria as indicated by delay of appearance of wilting symptoms increases in RWC of treated water stressed plants, and elevated antioxidant responses which were evident as elevated activities of enzymes such as catalase, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase as well as increased accumulation of antioxidants such as carotenoids and ascorbate. Results clearly indicate that the ability of wheat plants to withstand water stress is enhanced by application of these bacteria which also function as plant growth promoting rhizobacteria. Thus, osmo-tolerant PGPR strains could be used in field condition in order to mitigate salinity and water stress in crop plants

### 1. Introduction

Wheat is one of the most important cultivated cereals of the world. In different parts of India, productivity is affected by drought stress conditions. Besides direct water stress, increase in salinity also lowers osmotic potential leading to decreased water availability. Certain soil bacteria can help the plants to avoid or partially overcome a variety of environmental stresses. Plant growth promoting rhizobacteria, first defined by Kloepper and Schroth, (1978) include those bacteria, which, on inoculation into the soil, colonize the roots of plants and enhance plant growth. PGPR-elicited induced systemic tolerance (IST) help the growth of plants under abiotic stresses by producing various antioxidants, which result in the degradation of reactive oxygen species (ROS) (Figueredo et al., 2008).

The present study aims to isolate bacteria from rhizosphere of wheat as well another facultative halophyte, to select those bacteria with ability to grow in high salt medium and evaluate the different traits of such bacteria to utilize them as plant growth promoters as well as for alleviation of water stress. Besides, the study also aims to determine the biochemical mechanisms of induced systemic tolerance in wheat varieties.

### 2. Materials and Methods

### 2.1. Plant material

Six varieties of wheat (*Triticum aestivum* L.), namely Gayetri (GY), Mohan Wonder (MW), KW51, Ghandhari (GN), Kedar (KD) and PBW343 were collected and their viabilities were initially checked.

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### Induction of Plant Growth Promotion in *Camellia sinensis* by *Bacillus megaterium* and its Bioformulations

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Abstract: Plant growth promoting ability of Bacillus megaterium (TRS-4), isolated from rhizosphere of tea bushes, was tested on 5 varieties of tea- TV-18, TV-23, TV-25, TV-26 and T-17/1/154 in the experimental field and in potted condition. An increase in height, emergence of new leaves, branches and increase in leaf numbers as well as increase in leaf dry mass was observed following application of the bacterium. The experimental field was designed in randomized block design where three replicate plots with 10 plants /treatment for each variety was taken. In the field, the experimental plot of Tea Estate was arranged into 2-replicated randomized block design, with each treatment of variety T-17/1/154 having 100 bushes. Increase in number of leaves and number of branches were significant after 12 months of application in field but in potted plants, significant increase were obtained even after 2 months of application. Significant increase in accumulation of phenolics was also observed in tea plants. B. megaterium solubilized phosphate in vitro and in vivo. Following application of the bacterium, soil P content decreased, root and leaf phosphate increased and soil phosphatase activities were enhanced. Chlorophyll contents of leaves were enhanced by the application of B. megaterium. Bioformulations of the bacterium in saw dust, rice husk and tea waste were found to survive for more than 9 months in vitro with populations in the range of  $1 \times 10^{67}$  c.f.u/ml. The bioformulations were as effective as aqueous suspensions in plant growth promotion. There was no significant difference among the aqueous suspension or different bioformulations in increase in height and number of leaves after 2 months of application, indicating that the application of bioformulations could also effectively promote tea plant growth.

Key words: Bacillus megaterium % Growth promotion % Phosphate solubilization % Bioformulation

### **INTRODUCTION**

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the most important plantation crops in Darjeeling and Dooars regions, which are the tea growing regions of West Bengal, India. Over the years, productivity of the plant has been decreasing and one of the reasons for this has been attributed to the continuous use of huge quantities of chemicals in tea plantations. Hence, there is a pressing need in tea industry for utilizing either biological products completely or reducing the use of chemicals by supplementing with biological products in integrated management practices.

The rhizosphere, a zone very rich in nutrients, supports large microbial populations, which exert beneficial, neutral or detrimental effects on plant growth. Plant growth promoting rhizobacteria, first defined by Kloepper and Schroth [1], include those bacteria that are able to aggressively colonize plant roots and stimulate plant growth when applied to roots, tubers and seeds. Excess use of chemical fertilizers and fungicides has resulted in adverse impact on soil environment which in turn leads to loss in productivity and hence plant growth promoting rhizobacteria (PGPRs) are now finding increasing applications as biofertilizers [2]. The potential benefit of plant growth promoting rhizobacteria is not fully realized because of limitation like inconsistence performance at different location and season.

The mechanisms by which PGPRs can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanism may be direct i.e. production of growth hormones, phosphate solubilization, nitrogen fixation or indirect viz., suppression of deleterious microorganisms by

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ORIGINAL PAPER

# Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria

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Abstract Soil microorganisms with potential for alleviation of abiotic stresses in combination with plant growth promotion would be extremely useful tools in sustainable agriculture. To this end, the present study was initiated where forty-five salt tolerant bacterial isolates with ability to grow in high salt medium were obtained from the rhizosphere of Triticum aestivum and Imperata cylindrica. These bacteria were tested for plant-growth-promoting rhizobacteria traits in vitro such as phosphate solubilization, siderophore, ACC deaminase and IAA production. Of the forty-five isolates, W10 from wheat rhizosphere and IP8 from blady grass rhizosphere, which tested positive in all the tests were identified by morpholological, biochemical and 16SrDNA sequencing as Bacillus safensis and Ochrobactrum pseudogregnonense respectively and selected for in vivo studies. Both the bacteria could promote growth in six varieties of wheat tested in terms of increase in root and shoot biomass, height of plants, yield, as well as increase in chlorophyll content. Besides, the wheat plants could withstand water stress more efficiently in presence of the bacteria as indicated by delay in appearance of wilting symptoms increases in relative water content of treated water stressed plants in comparison to untreated stressed ones, and elevated antioxidant responses. Enhanced antioxidant responses were evident as elevated activities of enzymes such as catalase, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase as

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B. N. Chakraborty · P. L. Dey Immuno Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri 734013, West Bengal, India well as increased accumulation of antioxidants such as carotenoids and ascorbate. Results clearly indicate that the ability of wheat plants to withstand water stress is enhanced by application of these bacteria which also function as plant growth promoting rhizobacteria.

Keywords Wheat · PGPR · Drought · Antioxidants

### Abbreviations

| GY   | Gayetri                              |
|------|--------------------------------------|
| MW   | Mohan Wonder                         |
| GN   | Ghandhari                            |
| KD   | Kedar                                |
| RWC  | Relative water content               |
| PGPR | Plant growth promoting rhizobacteria |
| NA   | Nutrient agar                        |
| CAS  | Chrome Azurol S                      |
| CAT  | Catalase                             |
| SOD  | Superoxide dismutase                 |
| APOX | Ascorbate peroxidase                 |
| POX  | Peroxidase                           |
| GR   | Glutathione reductase                |
| PCR  | Polymerase chain reaction            |

#### Introduction

With rapid increase in population, projected to be 9.4 billion by 2050, worldwide food production needs to be significantly increased to gear up for meeting demands on food in the coming years. This is extremely important in the Indian context as India's population is predicted to reach a staggering 1.7 billion by 2050. On the other hand, various abiotic stresses affect plants and are causes of stagnation or reduction in crop productivity. Water stress is

### **RESEARCH ARTICLE**

### Influence of Serratia marcescens TRS-1 on growth promotion and induction of resistance in Camellia sinensis against Fomes lamaoensis

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Serratia marcescens (TRS-1), either as aqueous suspensions or in bioformulations of sawdust, rice husk and tea waste, promoted growth in tea seedlings as evidenced by increase in height, emergence of new leaves and branches, as well as increase in leaf biomass. Survival of *S. marcescens* in soil after application was determined by ELISA and Dot-Blot using PAb raised against the bacterium. *S. marcescens* solubilized phosphate *in vitro* and *in vivo*. Following application of the bacterium, soil P content decreased, root and leaf phosphate increased, and soil phosphatase activities were enhanced. The bacterium was antagonistic to a number of fungal pathogens *in vitro*. It also reduced brown root rot of tea caused by *Fomes lamaoensis*. Significant increase in phenolics, as well as peroxidase, chitinase,  $\beta$ -1,3-glucanase and phenylalanine ammonia-lyase, were observed in tea plants on application of *S. marcescens* alone or followed by *F. lamaoensis*.

**Keywords:** *Serratia marcescens*; growth promotion; defense enzymes; phosphate solubilization; acid and alkaline phosphatase; bioformulation

### Introduction

India is one of the major producers of tea in the world. Tea is produced from the leaves of *Camellia sinensis* (L.) O. Kuntze. It is a perennial and survives for more than 100 years. Over the years, in tea plantations, insecticides, chemical fertilizers and fungicides have been used indiscriminately to check crop losses. But such indiscriminate and excessive use of chemicals and cropping practices have brought about significant undesirable changes in soil microbial activity, and have also created the problem of pesticide residue in the leaves. Since the leaves are used as beverage, the problem of pesticide residue is very important, and hence, there is an urgent need to look for environmentally friendly, natural plant protectors.

Plant growth promoting rhizobacteria (PGPRs) are a common group of bacteria that can actively colonize plant roots and increase plant growth (Kloepper and Schroth 1978). These PGPRs can prevent the deleterious effects of phytopathogenic organisms from the environment. The mechanisms by which PGPRs can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanisms may be direct, i.e. production of growth hormones, phosphate solubilization, nitrogen fixation or indirect, such as suppression of deleterious microorganisms by siderophore production or secretion of antifungal metabolites (Kloepper 1993). However, in field conditions, the above traits may not be sufficient to account for the observed growth promotion. The biochemical or physiological changes induced in the host that are

activated by the PGPRs also lead to plant growth promotion and develop resistance capacity in the host against pathogens. Research over the past years has demonstrated that induced systemic resistance (ISR) can be a potential mechanism by which PGPR demonstrate biological disease control (Kloepper et al. 1996). ISR is dependent on colonization of the root system by sufficient numbers of PGPRs. Previous studies have shown that Serratia proteamaculans 1-102 promotes soybean-bradyrhizobia nodulation and growth, but the mechanism is unknown (Bai et al. 2002). Some biocontrol PGPB strains protect plants by activating gene encoding defense enzymes peroxidase, chitinase, phenylalanine ammonia-lyase,  $\beta$ -1,3-glucanase and others, involved in synthesis of phytoalexin (M'Piga et al. 1997).

Phosphorus is an essential nutrient for plants and an important component in cell metabolism. Its assimilation, storage and metabolism are of major importance to plant growth and development (Duff et al. 1994). Some common extracellular enzymes in soil are acid and alkaline phosphatases, produced by many organisms, which remove the phosphate molecule from organic compounds such as phospholipids and nucleic acids. Once the phosphate is cleaved it becomes soluble and can be taken up by the cell. This is an important activity because phosphate is often the limiting nutrient for microbial and plant growth in soil. Acid phosphatases catalyse non-specific hydrolysis of Pi from phosphate monoesters in pH ranges from 4–6 and help in the supply of phosphate in plants (Duff et al. 1994; Tabaldi et al. 2007).

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### Protection of Tea Plants Against Pest and Pathogen Through Combined Application of Pesticide and Plant Growth Promoting Rhizobacterium

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### Abstract

Pest and pathogen problems are major issues in tea estates which significantly reduce tea yields as well as quality. An integrated effect of a pesticide and a plant growth promoting rhizobacterium on insect attack, fungal disease and plant growth promotion in tea plants was investigated. Among five PGPRs isolated from tea rhizosphere, Bacillus megaterium (TRS4), Serratia marcescens (TRS1), Ochrobactrum anthropi (TRS2), Bacillus pumilus (TRS3) and Paenibacillus lentimorbus (TRS5), B. megaterium (TRS-4) promoted maximum growth of tea plants in field. In vitro tests were conducted to determine the tolerance of bacterium to acephate along with other insecticides- ethion 50EC and confidor, fungicides- hexaconazole, contaf 5E and calixin. Results revealed that B. megaterium could tolerate more than 100 times the recommended dose applied in field. For the integrated approach, a low dose (1:2000-0.5 mg/ml) of commonly applied acephate along with aqueous suspension of *B. megaterium* was tested. This combined application successfully controlled insect attack caused by Helopeltis theivora, resulting in improved growth of tea bushes and increase in number of leaves. Soil application of *B. megaterium* led to control of brown root rot of tea caused by *Phellinus noxius*, but the effect was much more pronounced when soil application of B. megaterium was supplemented with foliar spray of acephate. Besides polyphenol accumulation increases in defense related enzymes like phenyl alanine ammonia lyase, peroxidase, chitinase and  $\beta$ -1,3 glucanase in tea leaves were observed due to all the treatments. Chlorophyll content of leaves was enhanced along with an increase in isomers of catechins with the application of *B. megaterium*. This bacterium also solubilized phosphate in vitro and in vivo. Following application of the bacterium, soil P content decreased while root and leaf phosphate and soil phosphatese activities were enhanced. Sustainability of B. megaterium in soil was tested by PTA-ELISA and Dot immunobinding assay. Bioformulations of the bacterium in saw dust, rice husk and tea waste were found to survive for more than nine months in vitro with populations in the range of  $1 \times 10^{6-7}$  cfu/ml. The bioformulations were as effective as aqueous suspensions in plant growth promotion. Results of the present work clearly revealed that a combination of low dose of pesticide and a PGPR can be successfully used in tea plants for control of insect pest, fungal disease and overall plant growth promotion, without compromising on the flavour quality.

Key words: *Bacillus megaterium*, acephate, growth promotion, tea, brown root rot, defense enzymes, catechins, bioformulations.

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The rhizosphere, a zone very rich in nutrients, supports large microbial populations, which exert beneficial, neutral or detrimental effects on plant growth. Plant growth promoting rhizobacteria include (PGPR) those bacteria that are able to aggressively colonize plant roots and stimulate plant

growth when applied to roots, tubers and seeds. PGPRs have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric

nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Kloepper 1993). ISR (induced systemic resistance) could be a potential mechanism by which PGPRs demonstrate biological disease control (Kloepper et al 1996). ISR is dependent on colonization of the root system by sufficient numbers of PGPRs. Isolates of *Bacillus megaterium* have also been reported to produce antibiotics against several fungal pathogens (Jung and Kim 2003). Conversion of insoluble phosphates (both inorganic and organic) to a form accessible to the plants, like orthophosphate, is an important trait of a PGPR for

### **RESEARCH ARTICLE**



# *Bacillus megaterium* from tea rhizosphere promotes growth and induces systemic resistance in tea against *Sclerotium rolfsii*

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ABSTRACT: The objectives of the study was to determine how *Bacillus megaterium*, isolated from tea rhizosphere promotes the growth of tea plants as plant growth promoter, affects sclerotial blight disease and induces systemic resistance in tea plants. *Bacillus megaterium*, promoted growth of 5 varieties of tea, as evidenced by increase in height, emergence of new leaves, branches and increase in leaf numbers both in potted conditions and in the field. The bacterium was antagonistic to a number of fungal pathogens *in vitro* and reduced sclerotial blight of tea caused by *Sclerotium rolfsii*. Significant increases in phenolics along with increase in isomers of catechins as well as peroxidase, chitinase,  $\beta$ -1,3-glucanase and phenylalanine ammonia-lyase, were observed in tea plants on application of *B. megaterium* alone or following challenge inoculation with *S. rolfsii*. Survival of *B. megaterium* in soil was determined by ELISA and Dot-Blot using PAb raised against the bacterium. Enhanced expression of chitinase in *B.megaterium* treated leaves was confirmed by Western Blot and Dot Blot using PAb raised against chitinase. Besides, immunolocalization of chitinase in tea leaves was also done using the chitinase PAb. *B. megaterium* has been found to be a good plant growth promoter with the ability to increase growth of tea plants along with a reduction of sclerotial blight disease which acts both by direct and indirect mechanisms in the host.

Key words: Bacillus megaterium, chitinase, growth promotion, ISR, immunofluorescence, Sclerotium rolfsii

Rhizosphere is the habitat in which several biologically important processes and interactions take place. It is the zone of intense activity of various groups of microorganisms. The rhizosphere is the 1mm zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. This zone is where the majority of soil microorganism (bacteria and fungi) reside. Plant growth results from interaction of roots and shoots with the environment. The environment for roots is the soil or planting medium which provide structural support as well as water and nutrients to the plant. Roots also support the growth and functions of a complex of microorganisms that can have a profound effect on the growth and survival of plants. These microorganisms constitute rhizosphere microflora and can be categorized as deleterious, beneficial, or neutral with respect to root/plant health. Beneficial interactions between roots and microbes are common in rhizosphere and can be enhanced. Increased plant growth and crop yield can be obtained upon inoculating seeds or roots with certain specific root-colonizing bacteria-plant growth promoting rhizobacteria which can also prevent the deleterious effects of phytopathogenic organisms on the plants. The mechanisms by which PGPR can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanism may be direct i.e. production of growth hormones, phosphate solubilization, nitrogen fixation or indirect, viz. suppression of deleterious microorganisms by siderophore production or secretion of antifungal metabolites. However, in field conditions, the above traits

promotion. PGPR also lead to plant growth promotion and develop resistance capacity against pathogens by inducing biochemical or physiological changes in the host. Saharan and Nehra (2011) reported the use of plant growth-promoting rhizobacteria (PGPR) in various crop plants as growth promoters. Inoculation of crop plants with certain strains of PGPR at an early stage of development improved biomass production through direct effects on root and shoot growth. Some of the bacteria commonly used as PGPR include species of *Bacillus, Pseudomonas, Azotobacter, Azospirillum* etc. Previous studies have reported the use of some of these PGPR in tea for growth promotion and disease suppression (Chakraborty *et al.,* 2006, 2009, 2010).

may not be sufficient to account for the observed growth

The present study was undertaken to determine how *Bacillus megaterium* isolated from tea rhizosphere influences the growth of tea plants, affect sclerotial blight disease and induce systemic resistance in tea plants against this disease.

### MATERIALS AND METHODS

### **Test organisms**

**Bacterium:** Bacillus megaterium TRS 7 was isolated from the rhizosphere soil of tea bushes from Hansqua Tea Estate, West Bengal, India, and identified on the basis of morphological, microscopic and biochemical tests. The identity of the bacterium was further confirmed from the Plant Diagnostic and Identification Services, UK. Final molecular identification was done by 16SrDNA sequencing and sequence deposited to NCBI.

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# Serological and molecular detection of *Bipolaris sorokiniana* Sacc. causing Spot blotch disease of wheat

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Thirty five isolates of *B. sorokiniana* Sacc were collected from naturally infected wheat leaves grown in two locations in North Bengal. *B. sorokiniana* isolates exhibited variability in terms of morphology and virulence. Among these, one isolate (WH.PBW.IP.04) after completion of Koch's postulate was further identified by 18 S rDNA sequencing and also immunologically characterized. Fifteen wheat genotypes were screened for spot blotch resistance. Susceptible and resistant reactions were evaluated on the basis of appearance of infection on leaves following detached leaf inoculation technique and also in one month old potted wheat plants in glass house after 12,24,48,72 and 96 hr of inoculation. Out of ten susceptible genotypes CWL-6702 was found to be highly susceptible whereas CWL-6726(MUNAL 1) out of five genotypes showing most resistance against Spot Blotch. This was further confirmed by Dot-blot and ELISA using antibody of *B. sorokiniana*. Conidial germination was comparatively high in wheat leaves of susceptible genotypes. Increased accumulation of defense enzymes (chitinase, â-1,3 glucanase and phenyl alanine ammonia lyase) were observed in pathogen treated wheat leaves in respect to control.

Key words: Wheat, *Bipolaris sorokiniana*, spot blotch resistance, defense enzymes, Dot-blot, ELISA

### INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important food crop after rice in the world. A large number of wheat varieties have been released in the post-green revolution phase and new ones are continuously being added to the list every year. In India, Spot blotch has been a serious problem in north-eastern region as well as in north-western parts also (Singh and Srivastava, 1997). Due to wide spread losses, spot blotch is considered as the most important disease of wheat in the warm and humid regions of the world (Saari, 1998). In India, Spot blotch of wheat caused by *Bipolaris sorokiniana* Sacc. (syn. *Helminthosporium sativum* Pamm., King & Bakke) causes up to 36% loss under favourable conditions (Anonymous, 1997). The classification and identification of *Bipolaris* species is based on morphological characteristics. But recently, molecular biology techniques- PCR assays, RAPD, AFLP have been used to study the virulence and molecular diversity of *B. sorokiniana* isolates (Muller *et al*, 2005; Jahani *et al*, 2008; Zhong and Stefenson, 2001). Mondal (2000) reported that infected seeds, and soils infested either with conidial suspension or colonized grains may serve as potential sources for the survival of *B*.

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# Biological control of sclerotial blight of tea using arbuscular mycorrizal fungus and plant growth promoting rhizobacterium

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**ABSTRACT:** *Glomus fasciculatum*, one of the dominant arbuscular mycorrhizal fungus (AMF) associated with tea root colonization, was selected and maintained in maize plants. Besides AMF, *Bacillus amyloliquefaciens* TRS6 isolated from tea rhizosphere, which showed *in vitro* antagonism to a number of tea root rot pathogens as well as siderophore-producing and phosphate-solubilizing activities, was selected for utilization as bioinoculants in tea plants for improvement of health status. The bacterium was applied to the soil as aqueous suspensions, and in case of *G. fasciculatum*, soil was inoculated with its spores and in joint inoculations, the AMF was inoculated prior to the bacterium. Inoculation of rhizosphere of tea plants of six different varieties (TV-18, T-17, AV-2, T-78, UP-3 and UP-26) with any of two microorganisms increased growth of plants, but the most significant increase was obtained in dual application. Plant growth was measured in terms of increase in height, increase in number of branches and leaves. Similarly, sclerotial blight of tea, caused by *Sclerotium rolfsii*, was suppressed to certain extent by *G. fasciculatum* or *B. amyloliquefaciens*, but significant suppression occurred when *G. fasciculatum* and *B. amyloliquefaciens* were applied jointly. Polyphenolics and four major defense enzymes showed enhanced activities during disease suppression. Western blot of the enzyme extracts from control and all treated plants using PAb raised against chitinase revealed strong reaction when disease suppression was evident. Population of *S. rolfsii* in soil was also determined following immunological techniques using PAb raised against the pathogen. Results of ELISA and dot-blot revealed that application of *G. fasciculatum* and *B. amyloliquefaciens* significantly reduced *S. rolfsii* population.

Keywords: Disease control; induced resistance; Glomus fasciculatum; Bacillus amyloliquefaciens; sclerotial blight; tea

### Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is the major plantation crop of northeast India and forms the back bone of the economy of this region. It is a perennial and survives for more than 100 years. "Biological control against root diseases" and "plant growth promotion" are two important areas which are closely linked and have great impact on present-day agriculture. In tea plantations, with the reduction in the permissible levels of chemicals which can be used, there is urgent need for identification and selection of microbes which have the potential to control diseases as well as increase productivity.

Rhizosphere soil of plants contains a wide variety of microorganisms (bacteria, fungi and actinomycetes), some of which are beneficial, while others are harmful. Among the beneficial microorganisms, the following two groups are very important agriculturally: the mycorrhizal fungi and plant growth promoting rhizobacteria (PGPR). Mycorrhizal fungi colonize plant roots and extend the root system into the surrounding soil. Estimates of amounts of mycorrhizal filaments present in healthy soil

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are astonishing. Several miles of filaments can be present in less than a thimbleful of soil associated with vigorously growing plants. The relationship is beneficial because the plant enjoys improved nutrient and water uptake, disease resistance and superior survival and growth. AM fungal association protects the plants from soil-borne diseases and detoxifies soil contaminants of certain metals. AM fungi increase tolerance to heavy metals, salinity and drought.<sup>1,2</sup> AM fungi have been shown to increase the productivity of several cereals, pulses, oilseed crops, vegetable crops, medicinal plants and also ornamental plants. The AM fungi are obligate symbionts and not host-specific.<sup>3</sup> The spore count, root colonization, species diversity and dominant species vary with the region and soil nutrient conditions.<sup>4</sup> Many bacteria are known to be able to stimulate plant growth through direct or indirect interaction with plant root and these have been classified as plant growth-promoting rhizobacteria; first defined by Kloepper and Scroth.<sup>5</sup> The mechanisms by which PGPR can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanism may be direct, *i.e.* production of growth hormones, phosphate solubilization, nitrogen fixation or indirect, viz, suppression of deleterious microorganisms by siderophore production or secre-

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# Evaluation of Bacillus megaterium and Serratia marcescens and their bioformulations for promoting growth of Camellia sinensis

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ABSTRACT: A Bacillus megaterium (TRS-4) and Serratia marcescens (TRS-1), isolated from tea rhizosphere, enhanced growth of tea varieties TV-18, TV-23, TV-25, TV-26 and T-17/1/154 in the experimental field as well as in the nursery, as evidenced by increase in height, emergence of new leaves and branches. The experimental field was designed in randomized block design where three replicate plots with 10 plants/treat ment for each variety was taken. Treatment consisted of control + B. megaterium and control + S. marcescens. In field experiments, % increase in height and number of branches were significant after 6 and also 12 months of application, but in potted plants, significant increase were obtained even after 2 months of application. Both B. megaterium and S. marcescens solubilized phosphate in vitro and in vivo. Soil P content decreased due to the activity of these PSBs, while root and leaf phosphate contents showed an increase. Both acid and alkaline phosphatase activities in rhizosphere soil of all five varieties were enhanced following application of bacteria. The bacteria could survive in bioformulations of saw dust (6.1  $\times \log_{10}$  cfu/ml), rice husk (8.0  $\times \log_{10}$  cfu/ ml) and tea waste (6.98–7.2 ×  $\log_{10}$  cfu/ml) for more than 9 months in vitro. To test in vivo efficacy of these three formulations for plant growth promotion in the earlier-mentioned varieties, 2-month-old formulations were applied in potted soil at the rate of 100 g/pot. The bacterial inoculants in all three bioformulations were found to enhance growth of the test plants. Statistical analysis of variance (ANOVA) revealed that there was no significant difference among the aqueous suspension or different bioformulations though all of them significantly higher than control. However, all varieties did not show similar responses, with variations being observed in some cases. Survival of both bacteria in soil after application was determined by ELISA, Dot-Blot and Colony blot using PAb raised against the bacteria.

Keywords: Bacillus megaterium, Serratia marcescens, growth promotion, phosphate solubilization, acid and alkaline phosphatase, bioformulation, sustainability.

### Introduction

The thin layer of soil (about 1- to 2-mm thick) surrounding crop roots and the volume of soil occupied by roots is known as the rhizosphere. The sheer extent of crop roots in soil dictates that a significant portion of soil is actually within the rhizosphere (about 5–40% of soil in the rooting zone depending upon crop root architecture). This zone is where the majority of soil microorganism (bacteria and fungi) reside. These microorganisms constitute rhizosphere microflora and can be categorized as deleterious, beneficial or neutral with respect to root/ plant health. Rhizosphere microflora may vary greatly with the age of the plant and different localities of growth.

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important plantation crop grown in tropical agro climate. India is one of the major producers, consumers and exporters of tea. The excess use of chemical fertilizers and insecticides/ fungicides in tea plantations have led to global concern over side-effects and environmental pollution.<sup>1-3</sup> As against chemical fertilizers, the use of biological resources for growth improvement and disease control in tea has gained momentum in the last couple of decades.<sup>4-8</sup> The rhizosphere of tea bushes have some specific characteristics, which are associated with long lived nature of tea plants viz. negative rhizospheric effect, lowering of soil pH, antagonistic activities among microbial communities and dominance of certain species.<sup>9,10</sup>

Increased plant growth and crop yield can be obtained upon inoculating seeds or roots with certain specific root-colonizing bacteria – "plant growth promoting rhizobacteria". The use of beneficial microorganisms as biofertilizers and biocontrol agents has assumed importance in recent years, because these are environmentally safe and do not cause pollution.12

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# Molecular identification and immunological characterization of plant growth promoting rhizobacteria of *Camellia sinensis*

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**ABSTRACT:** Two rhizobacteria isolated from tea rhizosphere of Nagrakata and Hansqua tea estates were morphologically, biochemically characterized and finally identified as *Bacillus megaterium* (TRS 7) and *Serratia marcescens* (TRS 1). The BLAST query of 16S r DNA sequence of the isolates against GenBank database confirmed their identity. The sequences were deposited in NCBI, GenBank database under the accession Nos. JX 312687.1 and JN 020963.1 for *B.megaterium* and *S. marcescens* respectively. The identity of the isolates of *B. megaterium* (JX312687) and *S. marcescens* (JN 020963) were further confirmed by DGGE. Both the bacteria were able to produce IAA, volatiles, siderophores and solubilised phosphates *in vitro* but did not produce HCN and *B. megaterium* was non- chitinase producing strain. Both the bacteria showed antagonism to *Sclerotium rolfsii*. Effect of culture filtrate of *B. megaterium* and *S. marcescens* in inhibition of germination of sclerotia was noted. The bacterial sustainability in the soil was evaluated by ELISA, Western blot and Dot blot using their PAbs. *B. megaterium* and *S. marcescens* could successfully survive and multiply in tea rhizosphere even after three months of application.

KEYWORDS: PGPR; Bacillus megaterium; Serratia marcescens; DGGE; Sustainability; Tea.

### Introduction

Tea (Camellia sinensis (L.) O. Kuntze.) is the major plantation crop of North- East India and forms the back bone of the economy of this region. Excessive use of chemicals and the presence of residue in the leaves is a major concern for consumers. The use of beneficial micro-organisms as biofertilizers and biocontrol agents has become more important in recent years not only to improve plant growth and to manage plant diseases but also to avoid environmental pollution. As against chemical fertilizers, the use of biological resources for growth improvement and disease control in tea has gained momentum in the last couple of decades.<sup>1-4</sup> Therefore, there is an urgent need to select natural plant protectors. A safe and easy method to achieve this is to use the naturally occurring rhizobacterial strains which can effectively inhibit growth of pathogens and thereby protect the cultivated plants.

A long term study was carried out with a focus on rhizosphere microbiology of tea. Colonization by large

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ISSN: 0972-544X (print) © 2014 ISTS populations of antagonists and lowering the soil pH are the important characteristics associated with the tea rhizosphere.<sup>5-6</sup> The rhizosphere supports large and active microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth.

The microorganisms grow in close association with the plant and are referred to as rhizobacteria.7 They live at the expense of the plant, feeding on the nutrients released from the plant roots. The beneficial groups of microbes with the capacity to enhance plant growth by increasing seed emergence, plant weight and crop yields are designated as the plant growth promoting rhizobacteria (PGPR), first defined by Kloepper and Scroth.<sup>8</sup> The mechanisms by which PGPR can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanism may be direct i.e. production of growth hormones, phosphate solubilization, nitrogen fixation or indirect viz, suppression of deleterious microorganisms by siderophore production or secretion of antifungal metabolite.<sup>9</sup> Though different bacterial species have been investigated as biological control agents, the knowledge concerning the behaviour of these bacterial strains as antagonists and their genetic analysis is essential for effective use and the commercialization. Chakraborty et al.4 isolated a large number of rhizobacteria from the

# Plant growth promoting rhizobacteria mediated improvement of health status of tea plants

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Plant growth promoting rhizobacteria (PGPR) have immense potential application in sustainable agriculture as ecofriendly biofertilizers and biopesticides. The present study was undertaken to explore the potential of such microorganisms from the rhizosphere of tea [Camellia sinensis (L.) O. Kuntze] for the overall improvement in growth and productivity of tea, which is the most important crop of this region. Isolation and testing of bacteria for PGPR activities revealed that a large number of them showed such activities. Of which three were selected for various studies. The selected bacteria were Bacillus amyloliquefaciens, Serratia marcescens and B. pumilus. These bacteria showed positive PGPR traits in vitro, such as, phosphate solubilization, siderophore production, antagonism to pathogens and IAA production. 16S rDNA sequencing of the bacteria was done and their phylogenetic relationships determined. Under in vivo conditions, the PGPR enhanced the seedling growth of tea varieties in the nursery as well as in the field. Plant growth promotion was determined in terms of increase in number of leaves, their biomass and number of shoots. In order to determine the tolerance of bacteria to insecticides, in vitro tests were conducted, which indicated that PGPR could tolerate more than 100 times the concentration applied in the field. Sustainability of the applied bacteria in soil was tested by PTA-ELISA and Dot immunobinding assay using polyclonal antibodies raised against the PGPR. Certain bioformulations of the PGPR in talc powder, saw dust and rice husk also been prepared and their viability tested. The bacteria showed good survivability even up to 9 months of storage. Application of the PGPR led to enhancement in activities of defense related enzymes, such as, phenyl alanine ammonia lyase, peroxidase, chitinase and  $\beta$ -1,3-glucanase, in tea leaves. Total phenols also increased quantitatively. It is evident from the present study that application of PGPR in the soil lead to biopriming of the plants through induced systemic resistance and other mechanisms.

Keywords: Bacillus amyloliquefaciens, B. pumilus, Camellia sinensis, carrier based bioformulation, growth promotion, Serratia marcescens

### Introduction

Plant growth promoting rhizobacteria (PGPR), first defined by Kloepper and Schroth<sup>1</sup>, include those bacteria that are able to aggressively colonize plant roots and stimulate plant growth when applied to roots, tubers and seeds. PGPR have been reported to directly enhance plant growth by a variety of mechanisms like fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals, such as, phosphorus, and synthesis of phytohormones<sup>2</sup>. However, in field conditions, the above traits may not be sufficient to account for the observed growth promotion. The biochemical or physiological changes induced in the host that are activated by the PGPR also lead to plant growth promotion and develop resistance capacity in

Mobile: +91-9002002096; Fax: +91-353-2699001 E-mail: chakrabortyusha@hotmail.com the host against pathogens. Previous studies have shown that *Serratia proteamaculans* 1-102 promotes soybean—bradyrhizobia nodulation and growth, but the mechanism is unknown<sup>3</sup>. ISR (induced systemic resistance) could be a potential mechanism by which PGPR demonstrate biological disease control<sup>4</sup>. Isolates of *Bacillus megaterium* have also been reported to produce antibiotics against several fungal pathogens<sup>5</sup>.

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most important plantation crops in Darjeeling and Dooars regions, which are the tea growing regions of West Bengal, India. Productivity of tea plantations is decreasing and one of the reasons for this has been attributed to the continuous use of huge quantities of chemicals. In recent years, application of hexaconazole (RS)-2-2(2,4-dichlorophenyl)-1-(1,H-1,2,4-triazol-1-1yl) hexan-zol, an ergosterol biosynthesis inhibitor, has been common practice in tea cultivation areas<sup>6</sup>. Hence, there is a pressing need in tea industry for utilizing either biological produce completely or reducing the

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# Dual Application of *Bacillus pumilus* and *Glomus mosseae* for Improvement of Health Status of Mandarin Plants

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**Keywords:** *Citrus reticulata*, VAM, PGPR, phosphate solubilization, chitinase, peroxidase, β-1,3 glucanase

#### Abstract

Mandarin (Citrus reticulata) is an ancient commercial crop being cultivated in Darjeeling and Sikkim hills. Decline in general plant health and decreased fruit production as influenced by abiotic and biotic factors is a major threat to the cultivators in the region. Bacillus pumilus TRS 3, a potential plant growth promoting rhizobacterium, isolated from rhizosphere of plantation crop, which showed phosphate solubilization, siderophore production, IAA and volatile production in vitro, when applied to the rhizosphere of nursery grown mandarin seedlings, enhanced growth markedly. Plant growth promotion was determined in terms of increase in height of plants, number of leaves and biomass. B. pumilus also showed antagonism against fungal pathogens in vitro. In addition, screening of arbuscular mycorrhizal fungi from rhizosphere of mandarin plants yielded predominant association of Glomus mosseae, G. fasciculatum, G. intradices, G. versiforme, Gigaspora margarita, G. rosea, G. gigantea along with Acaulospora spinosa, A. bireticulata and Scutellospora sp. Maximum colonization by G. mosseae was evident which was selected for mass multiplication in maize plants. Mandarin roots were inoculated with G. mosseae alone and in combination with B. pumilus which was applied as soil drench. Both microorganisms increased growth of the plants but most significant increase was obtained when both were co-inoculated. Similarly, root rot of mandarin caused by Fusarium oxysporum, was suppressed to certain extent by B. pumilus or G. mosseae, but significant suppression occurred when G. mosseae was co-inoculated with B. pumilus. Three major enzymes involved in defense, chitinase,  $\beta$ -1,3 glucanase and peroxidase showed enhanced activities during disease suppression, which was also confirmed by immunological assays such as PTA-ELISA and Dot-blot. Observed plant health improvement and disease suppression in mandarin plants may be due to a combination of at least three mechanisms- direct inhibition of the pathogen in the soil, induction of resistance in the host or better nutrient availability.

#### INTRODUCTION

Citrus is the third priority fruit crop of India, after mango and banana. The most extensively grown citrus species in India is the common mandarin (*Citrus reticulata* Blanco, Family: *Rutaceae*, Order: Sapindales) in addition to sweet orange, lime and lemon. Darjeeling mandarin is the principal cash crop of Darjeeling hills situated between 27°13' and 26°27' North latitudes and between 88°53' and 87°59' East longitudes (Chettri et al., 2006). A marginal economy of the rural hill area is dependent on this crop. The present area under citrus cultivation is 0.8 million ha with an annual production of 8.8 million tonnes (National Research Centre for Citrus, Nagpur, India). Five different land races of common mandarin (loose jacket) are grown in India. They are Nagpur mandarin, Coorg mandarin, Khasi mandarin, Darjeeling mandarin in addition, one hybrid i.e. Kinnow mandarin grown in North-west India.

Rhizosphere soil of plants contains a wide variety of microorganisms, bacteria,

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# Induction of resistance in *Camellia sinensis* against *Sclerotium rolfsii* by dual application of *Rhizophagus fasciculatus* and *Bacillus pumilus*

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(Received 23 December 2014; accepted 14 August 2015)

*Rhizophagus fasciculatus* and *Bacillus pumilus*, isolated from rhizosphere of tea (*Camellia sinensis* (L.) O.Kuntze) bushes, were selected for the present study. Inoculation of tea bushes with any of the two micro-organisms increased growth of plants but significant increase was obtained in case of dual application. *B. pumilus* exhibited plant growth promoting rhizobacterial traits and antagonistic activity against *Sclerotium rolfsii in vitro*. Disease reduction following application of *R. fasciculatus* and *B. pumilus* was obtained when applied individually but joint application gave more significant results. A sharp increase was found in polyphenolic accumulation and activities of four defence enzymes which play a key role in disease suppression. Immunodetection of *S. rolfsii* and its population was significantly reduced owing to application of *B. pumilus* and *R. fasciculatus*.

Keywords: Rhizophagus fasciculatus; Bacillus pumilus; Sclerotium rolfsii; tea; growth promotion; disease suppression

### Introduction

Tea (Camellia sinensis (L.) O. Kuntze) is an important plantation crop grown in tropical agro climate. India is one of the major producers, consumers and exporters of tea. Being a perennial, it is susceptible to attacks by fungal and insect pests. Extensive use of fungicides and pesticides in tea plantations, combined with over doses of chemical fertilizers has caused the problem of pesticide residues in tea leaves, and has also deteriorated the condition of soil in several cases, leading to decrease in productivity. However, growing concerns over presence of chemical residues in tea leaves has intensified the search for alternatives. Rhizosphere is the habitat in which several biologically important processes and interactions take place and the micro-organisms that grow in the rhizosphere are ideal for use either as biological control agents and/or plant growth promoters. The use of beneficial micro-organisms as biofertilizers and biocontrol agents has assumed importance in recent years, because these are environmentally safe and do not cause pollution (Mathivanan et al. 2005). Among the beneficial micro-organisms, two groups are very important agriculturally – the mycorrhizal fungi and plant growth promoting rhizobacteria (PGPR). Enhanced plant nutrition by PGPR is mainly through increased phosphorous uptake by solubilisation of inorganic phosphate or mineralisation of organic phosphate.

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### Resistance in tea plants against root rot pathogens induced by arbuscular mycorrhizal fungi and plant growth promoters

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Tea, the most important plantation crop of North Bengal and Assam is valued for the beverage obtained from it and also the foreign exchange it brings in. Tea plant (Camellia sinensis), being a perennial harbors a number of microorganisms in its rhizosphere, both beneficial and harmful, some of which are responsible for causing a number of root rot diseases. The present study was undertaken to explore the potential of beneficial microorganisms from the rhizosphere of tea for growth improvement and biological control of diseases. The selected microorganisms which showed positive PGPR traits in vitro such as phosphate solubilization, siderophore production, antagonism to pathogens and IAA production were - Bacillus amyloliquefaciens, B. pumilus, B. megaterium and Ochrobactrum anthropi. The16S rDNA sequencing of the bacteria was done and their phylogenetic relationships determined. Glomus mosseae and G. fasciculatum, which were the dominant arbuscular mycorrhizal fungi (AMF) colonizing tea roots were selected for mass multiplication and application in nursery grown plants. Under in vivo conditions, the PGPR and AMF, applied either singly, or jointly, enhanced seedling growth of tea varieties in the nursery as well as in the field. Biocontrol of root diseases of tea caused by Sclerotium rolfsii, Phellinus noxius and Poria hypobrunnea was achieved by application of PGPR and AMF. Sustainability of the applied bacteria in soil was tested by PTA-ELISA and Dot immunobinding. Localization of AMF hyphae in root cells was determined by indirect immunofluorescence. Application of the PGPR and AMF led to enhancement in activities of defense related enzymes- phenyl alanine ammonia lyase, peroxidase, chitinase and  $\beta$ -1,3 glucanase in tea leaves. Total phenols also increased quantitatively along with increase in isoforms of catechins. It is evident from the results of the present study that application of PGPR and AMF in the soil lead to biopriming of the plants through growth promotion, induced systemic resistance and other mechanisms

Key words: Tea, AMF, PGPR, root diseases, defense enzymes

### INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the most important plantation crops in Darjeeling

and Dooars regions of West Bengal, which are the tea growing regions of West Bengal. The commercial value of the tea is due to its leaves, and more specially the tender leaves which are processed to produce the beverage yielding tea. Over the years, due to excessive use of chemicals, either as fertilizers or pesticides, productivity of the plant

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P R Verma Ph D student award-2015 Winner

Development of Immunological Formats for Detection of *Bipolaris sorokiniana* in Wheat Leaf and Strategies for Induction of Resistance against Spot Blotch Disease using Bioinoculants



#### **AP Chakraborty**

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### Abstract

Spot blotch of wheat caused by Bipolaris sorokiniana is one of the most important diseases of wheat. In the present study, thirty-five isolates of *B. sorokiniana* were collected from naturally infected wheat leaves. Among these, one isolate (BS29) after of completion of Koch's postulate was further identified by 18 S rDNA sequencing and also immunologically characterized. Serological formats using purified IgG of B. sorokiniana were developed for screening of resistance of 115 wheat germplasm against the pathogen following PTA-ELISA, Dot immunobinding assay. Simultaneously disease reactions were also evaluated in one month old field grown wheat plants and compared with serological data. Among the tested genotypes, CWL-6726 (MUNAL1) was found to be resistant, whereas, CWL (1-15), CWL22, CWL56, CWL57, CWL (63-69), CWL95, CWL96 were moderately resistant and CWL(16-21), CWL(23-43), CWL(44-50), CWL(51-55), CWL(58-62), CWL(70-89), CWL(90-92), CWL(97-100) were moderately susceptible. Conidial germination was comparatively high in wheat leaves of susceptible genotypes. Detection of B. sorokiniana in spot blotch infected wheat leaves was done by immuno-electron microscopy using PAb of pathogen. One of the highly susceptible genotype (CWL 6702) was further selected for induction of resistance using bioinoculants against B. sorokiniana. Talc based formulation of Bacillus methylotrophicus (NAIMCC-B 01492), a potent PGPR was applied both as seed treatment and foliar application, while wheat bran based formulation of Trichoderma asperellum (NCBIHQ 265418) and mass multiplied Glomus mosseae were used as soil application. Combination of all three formulations were found to be most effective in disease reduction. Time course accumulation of chitinase (CHT),  $\beta$ -1,3 glucanase (GLU) and phenyl alanine ammonia lyase (PAL) increased markedly in treated plants in comparison to healthy control following 12, 24,48, 72 and 96 h of challenge inoculation with B. sorokiniana. SDS PAGE analysis of soluble proteins and native PAGE of peroxizyme revealed increased band intensity in pathogen inoculated as well as bioinoculants treated and pathogen inoculated plants. Accumulation of p-coumaroylagmatine (phytoalexin) in treated and inoculated plants were analysed by HPLC. Immunolocalization of chitinase in bioinoculant treated and pathogen inoculated leaf tissue was further confirmed by transmission electron microscopy using PAb of chitinase and gold labelled conjugates.

Key words: Bipolaris soriokiniana, bioinoculants, induction of resistance, spot blotch.

**Citation:** Chakraborty AP, Chakraborty U and Chakraborty BN. 2016. Development of immunological formats for detection of *Bipolaris sorokiniana* in wheat leaf and strategies for induction of resistance against spot blotch disease using bioinoculants. *J Mycol Pl Pathol* 46(1): 21-37.

Wheat is the most important cereal crop after rice in India and major staple food of South Asian region countries. The warmer parts of the world are mainly affected by many diseases and among these, spot blotch caused by *Bipolaris sorokiniana* (Sacc.) (syn. *Helminthosporium sativum* Pamm., King & Bakke) is one of the most important disease in warm and humid regions of India and other South

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### **Research Article**

### Evaluation of streptomyces and non-streptomyces actinomycetes isolates for growth promotion in *Vigna radiata* and their use as biocontrol agent against *Sclerotium rolfsii*

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### Abstract

Two streptomyces (ARHS/PO26 and ARHS/PO27) and two non streptomyces (ARHS/Mn3 and ARHS/Mn7) actinomycetes isolates obtained from the rhizosphere soil of *Solanum tuberosum* and *Mangifera indica* were found to be phosphate solubilizers and showed antagonistic activity against *Sclerotium rolfsii*. Isolates ARHS/PO26 and ARHS/PO27 were identified morphologically and confirmed by the National Centre for Fungal Taxonomy, as *Streptomyces griseus* (NCFT 2578.08; NAIMCC-B-00916) and *Streptomyces griseolus* (NCFT 2579.08). ARHS/Mn 3 and *Streptomyces griseolus* (ARHS/PO27) could inhibit 68% and 59.7% growth of *Sclerotium rolfsii in vitro*. *In vivo* evaluation of the isolates ARHS/Mn 3, *Streptomyces griseolus* (ARHS/PO27) and *Streptomyces griseus* (ARHS/PO26) showed maximum growth promotion on *Vigna radiata* by enhancing key defense enzymes like chitinase,  $\beta$ -1,3-glucanase, phenylalanine ammonia lyase and peroxidase. The results emphasize the fact that soil actinomycetes could be used as potential biocontrol agents.

**Keywords:** Non-streptomyces Actinomycetes, *Streptomyces griseus, Streptomyces griseolus, Vigna radiata,* Growth promotion, Defense enzymes, *Sclerotium rolfsii* 

### Introduction

Mung bean or Green gram *Vigna radiata* (L.) Wilczek (svn: *Phaseolus aureus* Roxb.) constitutes the important group of grain legumes which form a major source of dietary proteins of high biological value, energy, minerals and vitamins (Taylor et al., 2005). Those who can not eat animal protein this plant belonging to the family Fabaceae or leguminosae is a good source of protein. However, the vield of mung bean is greatly reduced due to various factors of which diseases caused by fungi and viruses are of major concern (Satya et al., 2011). Now it has become necessary to find out ways of increasing yield and decreasing disease incidence in Vigna.

Streptomycetes are a group of actinobacteria which are part of the microbial flora of most natural substrates (Moustafa *et al.,* 1963) and mainly found in the rhizosphere of plants in association with other microorganisms like rhizobacteria and fungi. They utilize humic acid and other organic

matter in soil. In their natural habitat, such as forests, the actinomycetes interact in various ways with the higher plants (Lo et al., 2002). These organisms are part of PGPM or plant arowth promoting microorganisms. Streptomycetes affect plant health in various ways like by producing plant growth promoting hormones like IAA (Manulis et al., 1994), production of siderophores (Tokala et al., 2002) which influence plant growth or by protecting the plant against plant pathogenic microorganisms. It has been reported that secondary metabolites produced by some Streptomyces inhibit growth spp. of phytopathogenic fungi like Colletotrichum musae and Fusarium oxysporum (Taechowisan et al., 2005). Many of nonstreptomycete actinomycetes (NSA) taxa are therefore rarely reported in literature dealing with routine isolations of biocontrol agents and plant growth promoters from plant and soil. Seed-coating with powder formulation of the biocontrol agent was as effective as drench application of the fungicide, oxine benzoate (No-Damp), in controlling Rhizoctonia damping-off and superior to the commercial biocontrol agent, Streptomyces

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### **Research Article**

# Biochemical and Molecular Characterization of *Streptomyces* species isolated from Agricultural field of North Bengal and evaluation for growth improvement and suppression of sclerotial blight diseases of *Vigna radiata*

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### Abstract

Seventeen streptomycetesisolates were obtained from the rhizosphere of Solanum tuberosum. Their morphological attributes such as the colony colour, pigment production in culture media, colour of the aerial spore mass and a number of biochemical tests were executed to determine whether the isolates have the ability to hydrolyze starch, produce catalase and indole ring, which confirmed them as being streptomycetes. Among these, 15 isolates showed the ability of solubilizing phosphate by producing a transparent halo zone around the colony in Pikovskaya agar medium. In vitro antagonistic activity of some of these isolates against fungal pathogens (Fusarium graminarium, F. solani and Sclerotium rolfsii) were also confirmed by dual culture method. Three potent isolates were identified as Streptomyces tricolor (NCBI KX894280), S. flavogriseus (NCBI KX894281) and S. griseus (NCBI KX894282) by 16S rDNA technology. Phylogenetic analysis of the three isolates were conducted in MEGA4 and Streptomyces tricolor (NCBI KX894280), S. flavogriseus (NCBI KX894281) and S. griseus (NCBI KX894282) showed similarity with D44434.1 (S. tricolor), KC113491.1 (S. flavogriseus) and KJ623766.1 (S.griseus). In vivo evaluation of the growth promoting activity of the isolates on Vigna radiata revealed that S.tricolor (NCBI KX894280) and S. flavogriseus (NCBI KX894281) showed comparatively better growth promotion in comparison with the untreated control. These three Streptomycesisolates also enhances key defense enzymes like chitinase,  $\beta$  1-3 glucanase, and peroxidase in field condition which help in suppression of Sclerotial blight disease.

Key words: Gram positive actinomycetes, *Fusarium graminarium*, *F. solani* and *Sclerotium rolfsii*, *Streptomyces griseus*, *S. flavogriseus*, *S. tricolor*, 16S rDNA sequences

**Citation:** Ray P, Chakraborty AP and Chakraborty BN. 2016. Biochemical and Molecular Characterization of *Streptomyces* species isolated from Agricultural field of North Bengal and evaluation for growth improvement and suppression of sclerotial blight diseases of *Vigna radiata*. *Mycol Pl Pathol* 

Streptomycetesaremainly found in the rhizosphere of plants in association with other microorganisms like rhizobacteria and fungi. Streptomyce are a group of Actinobacteria. These are Gram positive filamentous soil inhabiting bacteria. Streptomyces sp. and other filamentous actinobacteria have been explored extensively over several decades for antibiotic production (Jog et al 2014). Actinomycetes are important producers of bioactive compounds and constitute a potential group of biocontrol agents. They have the capability to synthesize wide varieties of biologically active secondary metabolites such as antibiotics, pesticides, anti- parasitic compounds and enzymes like cellulase, xyla-nase, proteinase and chitinase (Sowndhararajan et al 2012).Some endophytic streptomycetes can also be used as biological control agents against plant pathogens.They utilize humic acid and other organic matter in soil. Patil et al (2010) isolated and characterized antagonistic actinomycetes- *Streptomyces tricolor* vh85 against *Rhizoctonia solani*. Dezfully and Ramanayaka

### **Research Article: KS Bilgrami Best Poster Award 2015–Winner**

# Biochemical Responses in *Sorghum bicolor* and *Triticum aestivum* to Spot Blotch Disease and Induction of Resistance by Plant Growth Promoting Rhizobacteria

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### Abstract

Two PGPR-*Bacillus altitudinus* and *B.megaterium* were tested to determine their efficacy in promoting induction of resistance in selected cereals (wheat and sorghum) against spot blotch. After 45 days of sowing, differences in growth rates between treated and untreated plants were evident. PGPR treated plants were challenge inoculated with *B. sorokiniana*. Disease development was computed alongwith analysis of different biochemical parameters. Increased accumulations of chitinase,  $\beta$ -1,3glucanase, phenylalanine ammonia lyase and peroxidase were observed in plants treated with the bioinoculants and challenge inoculated with pathogen when compared to untreated healthy plants. Application of PGPR also reduced stress signals like hydrogen peroxide and malonaldehyde in infected plants as a response to oxidative damage promoted by lipid peroxidat- ion under elevated free radical formation. Besides plant accumulate osmolyte proline in higher concentration in sorghum plants challenged with *B. sorokiniana* challenged with PGPR. Accumulation of antifungal phenolics in wheat and sorghum following bioinoculant treatment and challenge inoculation with pathogen was analysed using HPLC.

Key words: Defense enzymes, Bipolaris sorokiniana, phenolics, PGPR, spot blotch, Sorghum, wheat

**Citation:** Bhattacharjee Priyanka, Sarkar Jayanwita, Chakraborty AP, Chakraborty Usha and Chakraborty Bishwanath. 2017. Biochemical responses in *Sorghum bicolor* and *Triticum aestivum* to spot blotch disease and induction of resistance by plant growth promoting rhizobacteria. *J Mycol Pl Pathol* 47 (3): 252-261.

Sorghum [Sorghum bicolor (L) Moench] and wheat (Triticum aestivum L.) are the most important cereal crops in the world; and its wide range of other applications are now being explored with worldwide interest in renewable resources. In India, spot blotch has been a serious problem in both North-eastern region as well as in North-western parts. Bipolaris sorokiniana (Sacc.) Shoemaker (syn. Helminthosporium sativum teleomorph: Cochliobolus sativus), a hemibiotrophic pathogenic fungus known to cause spot blotch of sorghum and wheat. B. sorokiniana infect leaf, sheath and stem. Yield losses due to spot blotch vary from 16 to 33 per cent in wheat. B. sorokiniana is widely distributed in the areas where cereals are grown and forms a continuous genetic pool of isolates varying in virulence and aggressiveness to various cereals

and grasses. The infection process on the leaves usually occurs through natural wounding, stomata or with the use of an appresorium-like structure through the cell wall. The presence of other hosts plays an important role in disease epidemic. The primary inoculum of *B. sorokiniana* comes from several sources such as weed hosts, soil, crop debris which enhances the disease level. Therefore, the present investigation was undertaken to determine the biochemical response of sorghum and wheat to the foliar fungal pathogen *B. sorokiniana* and also possible induction of resistance through the use of two bioinoculants.

### **Materials and Methods**

**Plant material.** Locally cultivated wheat variety PBW 343 and Sudan grass of sorghum were

<sup>\*</sup>At 37th Annual Conference and National Symposium of ISMPP, RAU, Pusa (Samastipur), Bihar, 4–6 Apr 2016.

# *Bacillus safensis* from wheat rhizosphere promotes growth and ameliorates salinity stress in wheat

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*Bacillus safensis* (W10) from wheat rhizosphere which could grow in medium with high concentration of sodium chloride selected for this study showed positive response in *in vitro* plant growth promoting rhizobacteria (PGPR) tests such as phosphate solubilization, siderophore production, indole acetic acid (IAA) production and 1 aminocyclopropane-1-carboxylase (ACC) deaminase activity. The growth of six cultivars of wheat under normal as well as salt-stressed conditions was promoted by this bacterium also enhanced the tolerance of wheat cultivars to salinity. Activities of antioxidant enzymes were enhanced due to salinity, but not by bacterial application, while those of defense enzymes were significantly enhanced due to application of W10 alone or with NaCl treatment. Microarray and bioinformatic analysis for comparing gene expression in seedlings under salinity stress against those under salinity stress along with bacterial application revealed an up-regulation of 3731 genes and down-regulation of 6312 genes. Some of the up-regulated genes included those for expansins, endotransglucoslylase/hydrolase, sulphur-rich thionin, S-adenosylmethionine decarboxylase precursor and metallothionines while down regulated ones included those for flavanone hydroxylase, oxalate oxidase, protein phosphatase etc. Probably, salinity stress in wheat is alleviated mainly not through enhanced antioxidant systems but up-regulation of several other genes involved in tolerance to salinity as well as defense against biotic stress.

Keywords: Wheat, Bacillus safensis; salinity; antioxidative enzymes; defense enzymes; microarray

### Introduction

Various abiotic stresses such as drought, salinity and temperature fluctuations affect plants and are causes of stagnation or reduction in crop productivity. Salinization is recognized as the main threat to environmental resources in many countries and affects almost one billion hectares worldwide<sup>1</sup>. Natural salinity is the result of long-term natural accumulation of salts in the soil or in surface water. Secondary salinity results from irrigation and is widely responsible for increasing the concentration of dissolved salts in the soil profile that impairs plant growth and will result in abandoning agricultural lands<sup>2,3</sup>. Many of the most cultivated and widely used crops (cereals, horticultural crops, etc.) are susceptible to salt stress (> 4 dsm<sup>-1</sup>), and their productivity is reduced due to improper nutrition of the plant<sup>4-5</sup>. Wheat an important cereal crop, ranks first in acreage as well as production among the crops of the world.

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Salinity exerts negative influences on its production and reduces the yield (Ghane et al)<sup>6</sup>. Kumar et al<sup>7</sup> selected eight genotypes of wheat to evaluate effect of salinity on germination, growth and yield. Lower salinity (3 dsm<sup>-1</sup>) did not affect the germination, growth and yield. Higher salinity levels reduced germination, growth and yield attributing parameters. Genotypes K9006, K8434, KRL1-4, K88 and HD2733 showed hardness against higher levels of salinity. In a study by Akram et al<sup>8</sup> it was reported that increased levels of NaCl salinity (2.5, 10, 15, 20 dsm<sup>-1</sup>) showed effects on yield components and growth parameters of salt tolerant (234/2), medium responsive (243/1) and sensitive (Fsd-83) accessions/ varieties of wheat (Triticum aestivum L.). Salinity reduced spike length, number of spikelets per spike, number of grains per spikelet, 100 grain weight and grain yield per plant. Shamsi and Kobraee9 showed that number of grains per spike, number of grain per spikelet, number of fertile spikelet, number of tillers, 1000 grain wheat, plant height, grain yield, biological yield, harvest index and relative

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FULL-LENGTH RESEARCH ARTICLE



# **Biochemical Responses of Wheat Plants Primed** with *Ochrobactrum pseudogrignonense* and Subjected to Salinity Stress

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**Abstract** A large number of bacteria were isolated from the rhizosphere of *Imperata cylindrica*, a facultative halophyte, and the most tolerant one with an ability to grow under 10% sodium chloride in vitro was selected and designated as IP8. This was identified to be *Ochrobactrum pseudogrignonense* on the basis of 16SrDNA analysis and showed positive plant-growth-promoting traits in vitro. Growth of wheat was enhanced by the bacterium under both non-stressed and salt-stressed conditions. Alleviation of salt stress by the bacterium was evident by the biochemical responses such as reduction in hydrogen peroxide accumulation as well as enhanced proline accumulation and antioxidative mechanisms. Activities of peroxidase, catalase, ascorbate peroxidase, and glutathione reductase were enhanced after salinity treatment, but that of superoxide dismutase was not. Treatment with IP8 and sodium chloride showed enhanced activities, and when compared to sodium chloride alone, peroxidase showed further up-regulation in some cultivars and superoxide dismutase also showed significantly increased activities. Salinity had no effect on activities of defense enzymes in general. Activities of defense enzymes were significantly enhanced by IP8 alone or in addition to salinity treatment. Microarray analysis revealed that 282 genes were down-regulated and 6022 up-regulated after application of *O. pseudogrignonense* under salt stress induction by 200 mM sodium chloride. Among the up-regulated genes were those of peroxidase, phenylalanine ammonia lyase, chitinase, glucanase, as well as germin-like proteins, histone H2B, and sulfur-rich thionin-like proteins. Genes for ascorbate peroxidase, lipid transfer proteins, and salt stress responsiveness were among the down-regulated ones.

Keywords Sodium chloride · Stress alleviation · Antioxidants · Plant-growth-promoting rhizobacteria

### Introduction

Various abiotic stresses affect plants and are causes of stagnation or reduction in crop productivity. On the other hand, there is a need for a significant increase in food productivity worldwide, since it is projected that by 2050, world population would be a staggering 9.4 billion. Among

U. Chakraborty ucnbu2012@gmail.com the abiotic stresses, drought, salinity, and temperature fluctuations occupy a prominent position as affecting agriculture. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within next 25 years and up to 50% by the middle of the twenty-first century [32]. Agricultural lands that have been heavily irrigated are highly saline. This is because, in dry areas which are dependent solely on irrigation, water loss occurs through a combination of both evaporation as well as transpiration and as a result, the salt delivered along with the irrigation water gets concentrated, year-by-year in the soil. This leads to huge losses in terms of arable land and productivity as most of the economically important crop species are very sensitive to soil salinity. Basic physiology of water stress and salinity is similar since excessive salinity decreases available water in the

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# **15** Role of Microorganisms in Alleviation of Abiotic Stresses for Sustainable Agriculture

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#### Abstract

Abiotic stresses affect plants in different ways and are causes of reduction in crop productivity. In order to increase crop productivity it becomes necessary to evolve efficient low-cost technologies for abiotic stress management. Soil microorganisms, surviving in the soil under extreme conditions, have shown great properties, which, if exploited can serve agriculture for increasing and maintaining crop productivity. While it is well established that beneficial soil microorganisms can promote growth and increase productivity through mechanisms such as nutrient mobilization, hormone secretion and disease suppression, it is also becoming increasingly clear that their effects maybe more far-reaching. Several studies have reported that soil microorganisms may have mechanisms for alleviation of abiotic stresses in plants such as water and temperature stress, salinity, heavy metals etc. Some of these include tolerance to salinity, drought (Azospirillum sp., Pseudomonas syringae, P. fluorescens, Bacillus sp.) and nutrient deficiency (Bacillus polymyxa, Pseudomonas alacaligenes). Other than bacteria, salinity- and drought-tolerant isolates of Trichoderma harzianum and the effect of other strains of Trichoderma in amelioration of such abiotic stresses have also been reported. Arbuscular mycorrhizal fungi (Glomus mosseae, G. etunicatum, G. intraradices, G. fasciculatum, G. macrocarpum, G. coronatum etc.) help in alleviating abiotic stresses in different crops by enhancing nutrient uptake (phosphorous, nitrogen, magnesium and calcium), biochemical (accumulation of proline, betaines, polyamines, carbohydrates and antioxidants), physiological, molecular and ultra-structural changes. In the present chapter, we attempt an overview of current knowledge on how plant-rhizobacteria, plant-Trichoderma as well as plant-mycorrhiza interactions help in alleviating abiotic stress conditions in different crop systems, which can be used for sustainable agriculture.

### 15.1 Introduction

Plants, which remain rooted to the soil, are subjected to varying types of abiotic stresses throughout the course of their life span and they have to develop mechanisms for coping with such stresses in order to survive. Agriculture is extremely sensitive to environmental changes such as high and low temperatures, drought, flooding, salinity, freezing, change in pH, strong light, UV and heavy metals. Such adverse environmental conditions have a negative impact on crop production, which has the potential to become a major problem for food security, particularly in tropical regions. Abiotic stress management in plants

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## PLANT GROWTH PROMOTING RHIZOBACTERIA : DIVERSITY, MECHANISMS OF ACTION AND PERSPECTIVES IN AGRICULTURE

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KEY WORDS : PGPR, Bacillus, Pseudomonas, Paenibacillus, Biocontrol

### ABSTRACT

Among the large group of microorganisms in the soil or in the rhizosphere, plant growth promoting rhizobacteria (PGPR) are a common group that can actively colonize plant roots and increase plant growth through different mechanisms. These do not belong to a single group, but have great diversity, and some of the common PGPR which have been identified so far belong to genera such as Bacillus, Pseudomonas, Paenibacillus and many others. Though a large number of rhizospheric bacteria have been isolated and identified as potential PGPR, this is just the tip of the iceberg. More than 95% bacteria still remain uncluturable and need techniques other than usual isolation if they are to be unravelled. An alternative approach, is the analysis of genes within environmental samples. These genes may be functional genes, i.e. those coding for proteins performing particular metabolic reactions of relevance to ecosystem processes. However, most applications have analysed genes encoding the small subunit (SSU) of ribosomal RNA. PGPR act through different mechanisms such as phosphate solubilization, phytohormone production, nitrogen fixation, ACC deaminase production or indirectly through disease suppression leading to crop improvement. Biocontrol may again be through direct inhibition of pathogen in soil or induction of systemic resistance in the host. However, in spite of successful demonstrations of the use of PGPR in laboratory and green house conditions, their use in large scale agriculture is still limited. Hence for maximum benefits, such microorganisms need to be used in combination with low doses of chemical fertilizers and/or pesticides in integrated management. On the other hand, while introducing microorganisms into the field, it is also essential to know how they will interact with the resident microbial community. The selection of the appropriate strains for the enhanced efficiency of PGPR under different conditions is of significance.

### **INTRODUCTION**

The rhizosphere can be defined as any volume of soil specifically influenced by plant roots and/or in association with roots and hairs, and plant-produced material (138, 35). This space includes soil bound by plant roots, often extending a few mm from the root surface and can include the plant root epidermal layer (35). Plant exudates in the rhizosphere, such as amino acids and sugars, provide a rich source of energy and nutrients for bacteria. resulting in bacterial populations greater in this area than outside the rhizosphere. Most rhizosphere organisms occur within 50 µm of root surface and populations within 10 um of root surface may reach 109-1012 microbial cells g 1 soil. Despite large numbers of bacteria in the rhizosphere, only 7–15% of the total root surface is generally occupied by microbial cells (168). The functions of soil biota are central to decomposition processes and nutrient cycling. Therefore, major microbial activity is confined to the 'hot-spot', i.e. aggregates with accumulated organic matter, rhizosphere (134, 168). The region around the root, the rhizosphere, is relatively rich in nutrients, due to the loss of as much as 40% of plant photosynthates from the roots (133). Consequently, the rhizosphere supports large and active microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth. The importance of rhizosphere microbial populations for maintenance of root health, nutrient uptake, and tolerance of environmental stress is now recognized (34). These beneficial

216

Chapter 5

# Functional and Genetic Diversity of Bacterial Isolates from Soil and Utilization of their Beneficial Traits for Crop Improvement

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### ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are a common group of bacteria that can activelycolonize plant roots and increase plant growth. They are beneficial soil bacteria, which may facilitateplant growth and development both directly and indirectly. Since PGPR function through a number of mechanisms they exhibit a wide variety with respect to their functions. However there are many bacteria which exhibit not one, but different mechanisms of action. Functional diversity can be distinguished on the basis of their different roles such as N<sub>2</sub>-fixing, phosphorus-solubilizing, siderophore-producing, HCN producing, antibiotic producing, heavy-metaltolerant and hormone producing bacteria in growth promotion and disease reduction. An increasing interest has emerged with respect to the importance of microbial diversity in soil habitats. The extent of the diversity of microorganisms in soil is seen to be critical to the maintenance of soil health and quality, as a wide range of microorganisms is involved in important soil functions. Genetic diversity can be based on functional genes and conserved gene sequence among soilbeneficialbacteria (PGPR). Future perspectives of PGPR have been highlighted thatoffer an environmentally sustainable approach to increase crop production and health. The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides, and supplements. Application of molecular tools in agriculture is also enhancing our ability to understand and manage therhizosphere and will lead to new products with improved effectiveness. We have isolated a number of bacteria from soils of North Bengal region showing PGPR. characteristics such as phosphate solubilization, siderophore production, antagonism against pathogens etc and these have also been shown to have the ability of promoting growth in vivo. The molecular diversities of these bacteria have also been determined.

Key words: Plant growth promoting rhizobacteria (PGPR); Growth promotion; disease reduction; Molecular diversity; crop health improvement

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