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Systemic infestation of *Serratia entomophila* AB2 through plant tissue inferred protection against insect pest and fungal pathogens

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***Serratia entomophila*, a Gram negative non spore forming bacteria, is commercially exploited as biocontrol agent of pasture pest, grass grub (*Costelytra zealandica*, Coleoptera) in New Zealand as soil inoculants. As an exception, the strain *S. entomophila* AB2 used in this study is having insecticidal property against pod borer (*Heliothis armigera*, Lepidoptera), a phyllospheric pest. Expermental results showed high rate of mortality when the pests were fed with shoot portion of ground nut developed from bacteria treated seeds. Antifungal property of *S. entomophila* AB2 was recorded when tested *in vivo* using different parts of plant developed from bacteria treated and untreated seeds. In the present study, the systemic infestation was understood by isolation and enumeration of the inoculant from different plant tissues in specific medium and an increment in number within the tissues was also observed. This is the first report of systemic spreading of *S. entomophila* in plant parts to provide protection against insect and fungal pests.**

Key words: Systemic infestation, biocontrol, insect pest, plant pathogenic fungi, *Serratia entomophila.*

INTRODUCTION

As a natural biocontrol agent against grass grub *Costelytra zealandica* (Coleoptera), *Serratia entomophila* was first reported by Grimont et al. (1988) and thereafter by Jackson et al. (1992), Glare et al. (1993), Nunez-Valdez and Mahanty (1996), Hurst et al. (2007) and Young et al. (2009). A Mexican strain (*S. entomophila* Mor4.1) was also found to be active against another white grub, *Phyllophaga blanchardi* (Coleoptera) by Nunez-Valdez et al. (2008). In addition, *S. entomophila* M6 demonstrated further agricultural potentiality of neutralising heavy metals (Ji et al., 2012). The experimental isolate, *S. entomophila* AB2 reported from epizootic *Heliothis* sp. (Lepidoptera) could reciprocate for pesticidal and nutrient solubilisation property (Chattopadhyay et al., 2011, 2012).

Rhizosphere is a natural repository of diversified microorganisms. The pathogenic strains of pasture pests make them effective for pest control by rhizospheric application. However, *S. entomophila* AB2 is known to attack Lepidopteron pest in the phyllosphere (Chattopadhyay et al., 2012). It is important to study whether, the rhizospheric application of the strain AB2 could provide an effective protection against Lepidopteron pest.

Therefore, the main objective of the present study was to find out the efficacy of rhizospheric application of *S. entomophila* AB2 to control pest and fungal attack at phyllosphere in laboratory condition and the extent of bacterial dispersal inside the plant parts.

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MATERIALS AND METHODS

Experimental organism used

Bacterial strain *S. entomophila* AB2, was previously isolated, characterized and identified by our team and currently obtained from Prof. Sukanta K. Sen, Microbiology Division, Department of Botany, Visva-Bharati University, India. The 16S rRNA gene sequence was registered to Gene Bank (Gene Bank accession no. GU370899). The isolate was reported earlier for its pesticidal property against lepidopteran pest (Chattopadhyay et al., 2011, 2012).

Bacterial culture was maintained as 50% glycerol stock at -20°C in brain heart infusion agar (BHI-agar, Hi-media, India). The working strain was grown in shake flask containing 100 ml of broth (4 g sugar, 1 g yeast extract, 0.2 g urea and 0.2 g NPK; pH 7.1). Fermentation was carried at 28°C for 30 h in an orbital shaker (Scigenics Biotech, India) at 240 rpm.

Fungal phytopathogens used

The phytopathogens, *Aspergillus flavus* MTCC 1973, *Candida albicans* MTCC 183 and *Fusarium oxysporum* MTCC 1753 were maintained in recommended media (http://mtcc.imtech.res.in/catalogue) and cultured using Potato Dextrose Agar (PDA, HiMedia, India) for *in vitro* and *in vivo* assessment of antifungal activity. For *in vivo* assessment of antifungal activity 8 mm discs were prepared from 5 day old cultures.

Rearing of bollworm

Heliothis armigera larvae were obtained from the field and maintained on a commercial diet (IM002, Hi-Media, India) in plastic vials (Tarsons, India) at constant ambient temperature (Chattopadhyay et al., 2011). In order to obtain a homogeneous mass of the test insects, the larvae were reared till emergence of adults. After rearing for two generations, 5-day-old larvae were used for bioassay (Chattopadhyay et al., 2011).

Bacterial treatment of seeds

Surface sterilized seeds of *Arachis hypogaea* var. Koushal G201 were treated with *S. entomophila* AB2 (2 ml of 10^9 cfu ml^{-1}seed-1) and air dried for 1 h in a laminar cabinet at RT (28±2°C). After treatment, the seeds were taken with a sterile forcep and placed on moistened blotter paper in Petri dishes (10 seeds/plate). The seeds were incubated at RT (28±2°C) for 10 days, maintaining the standard moisture condition (40%).

Efficacy against test insect

For bioefficacy test against Lepidopteron pest, the roots and shoots of germinated seeds were cut into bits of 1 cm maintaining sterility. To record the mortality rate after 72 h, experimental larvae (5-day old) were distributed into 5 separate batches, each containing 30 larvae but in separate vials with 3 g of feed at constant ambient temperature (28±2°C). Batch No. 1 was fed with commercial diet (IM002, Hi-Media, India). Batch No. 2 and No. 3 were fed with shoot bits and root bits (respectively) derived from 10 days old seedling of bacteria untreated seed. Similarly, Batch No. 4 and No. 5 were fed with shoot bits and root bits (respectively) derived from bacteria treated seed.

Efficacy against fungal phytopathogens

In vitro assessment of antifungal efficacy of *S. entomophila* AB2 was carried out with test fungi through bore well method using PDA. The inocula, *S. entomophila* AB2 (48 h old) and test fungi (168 h old) were put oppositely and 25 mm away from the periphery of the Petri plates, in three replicates (Dennis and Webster, 1971). Petri plates inoculated with the fungal phytopathogen alone served as the control. The plates were incubated at 28±2°C. After 72 h of incubation, the inhibition of mycelial growth (%) was calculated according to the study of Vincent (1927).

In vivo assessment of antifungal efficacy was carried out with seedling parts (10 days old) derived from bacteria untreated seed and bacteria untreated seeds. Roots/shoots were separated and cut in to bits as described at specific distance (2, 4, 6 and 8 cm away) from seed coat. The bits of root/shoot from bacteria treated/untreated seeds were surface sterilized and placed on Nutrient agar medium and incubated at 28°C for overnight. After incubation, 8 mm discs of test fungi were placed just opposite the plant parts, incubated at room temperature for five days and the inhibition zone, if any, was measured.

Assessment of systemic infestation into plant parts

Roots/shoots/leaves were separated at regular interval (day 4, 6, 8 and 10) from seedlings (derived from bacteria treated and untreated seeds). The roots and shoots were cut as stated and ground (1 g) individually in a sterile mortar-pestle and suspended in 9.9 ml sterile distilled water in tubes, maintaining asepsin. The tube containing sample was vortexed for 30 s and placed in slanting position in an orbital shaker (10 rpm) for 1 h. For enumeration of *S. entomophila* AB2, serially diluted suspensions were plated onto caprylate thallous agar (CTA) medium (O'Callaghan et al., 2002) supplemented with antibiotic Ampicillin (A). The colony count was taken against the UV lamp (Kishore et al., 2005).

Statistical analysis

Each experiment was performed at least for 3 times unless stated otherwise. Standard deviation for each treatment was determined. The experimental data were statistically analyzed using box plot analysis. Pesticidal activity evaluated, through pest scouting and evaluation of mortality rate, on the basis of severity of infestations (Amer et al., 1999).

RESULTS AND DISCUSSION

Efficacy of *S. entomophila* AB2 against pod borer

In vivo pesticidal activity was measured between 3.33-90.0% against the test pest. High mortality rate was recorded in Batch No. 4 (90%) and No. 5 (87%) of experimental larvae, fed with roots/shoots of bacteria treated seeds in comparison to Batch No.2 (7%) and No. 3 (7%) where experimental larvae were fed with roots/shoots of bacteria untreated seeds. While the lowest mortality rate was recorded in Batch No. 1 of experimental larvae fed with commercial diet.

These results clearly indicate that the shoot bits and root bits derived from bacteria treated seeds are with sufficient inoculants (*S. entomophila* AB2 population) to

Table 1. Mortality (%) of pod borer (*H. armigera* larvae) in feeding experiment.

Batch No.	Treatment	Pest mortality (%)
1	Commercial diet	3.33 ± 0.03
2	Plant shoot from bacteria untreated seed	6.66 ± 0.03
3	Plant root from bacteria untreated seed	6.66 ± 0.03
4	Plant shoot from bacteria treated seed	86.66 ± 0.06
5	Plant root from bacteria treated seed	90.00 ± 0.06

Table 2. *In vitro* efficacy of *S. entomophila* AB2 against fungal phytopathogens.

***S. entomophila* AB2 (1.5×10^8 ml^{-1})**	**Inhibition zone (dia in mm)**		
	A. flavus	***C. albicans***	***F. oxysporum***
50 µL	14.50±0.25	19.50±0.25	17.00±0.25
100 µL	18.75±0.25	23.75±0.25	20.75±0.25
150 µL	21.25±0.25	29.25±0.25	24.25±0.25
200 µL	23.25±0.25	32.25±0.25	28.75±0.25

Table 3. *In vivo* efficacy of *S. entomophila* AB2 against fungal phytopathogens

Plant part distance (in cm from seed coat of 10 day seedling)	**Phytopathogens**	**Inhibition zone (dia in mm)**	
		Shoot	**Root**
2	***A. flavus***	15.40±0.25	19.25±0.25
4		13.65±0.25	17.80±0.25
6		11.60±0.25	16.25±0.25
8		08.80±0.25	14.45±0.25
2	***C. albicans***	19.80±0.25	24.65±0.25
4		17.50±0.25	22.80±0.25
6		14.70±0.25	20.45±0.25
8		12.25±0.25	19.80±0.25
2	***F. oxysporum***	18.20±0.25	22.55±0.25
4		16.45±0.25	20.00±0.25
6		13.75±0.25	17.45±0.25
8		11.45±0.25	15.25±0.25

impart pesticidal activity to test larvae (Table 1). Availability of *S. entomophila* from phyllosphere region of tomato and banana are reported (Akutsu et al., 1993; Riveros et al., 2002).

Efficacy of *S. entomophila* AB2 against phytopathogenic fungi

In *in vitro* experiment, *S. entomophila* AB2 exhibited high spectrum of antifungal activity in a concentration dependent manner (Table 2) but found more antagonistic to *C. albicans*.

In *in vivo* experiment also, the plant parts from bacteria treated seeds showed positive activity against the test pathogens. Results (Table 3) clearly showed that *S. entomophila* AB2 was more antagonistic to *C. albicans*, as found from the *in vitro* study (Table 2).

Several reports are available about the antagonistic effect of *S. marcescens* against various fungal pathogens (Akutsu et al., 1993; Iyozumi et al., 1996; El-Tarabily et al., 2000; Someya et al., 2000). The rhizobacterial strain *Serratia marcescens* 90 to 166 mediates induced systemic resistance (ISR) to fungal, bacterial, and viral pathogens (Press et al., 1997). Hence, fungal antagonistic property, substantiated with pest larval mortality, while fed with different parts of test plant, supports the theory of systemic infestation by *S. entomophila* AB2.

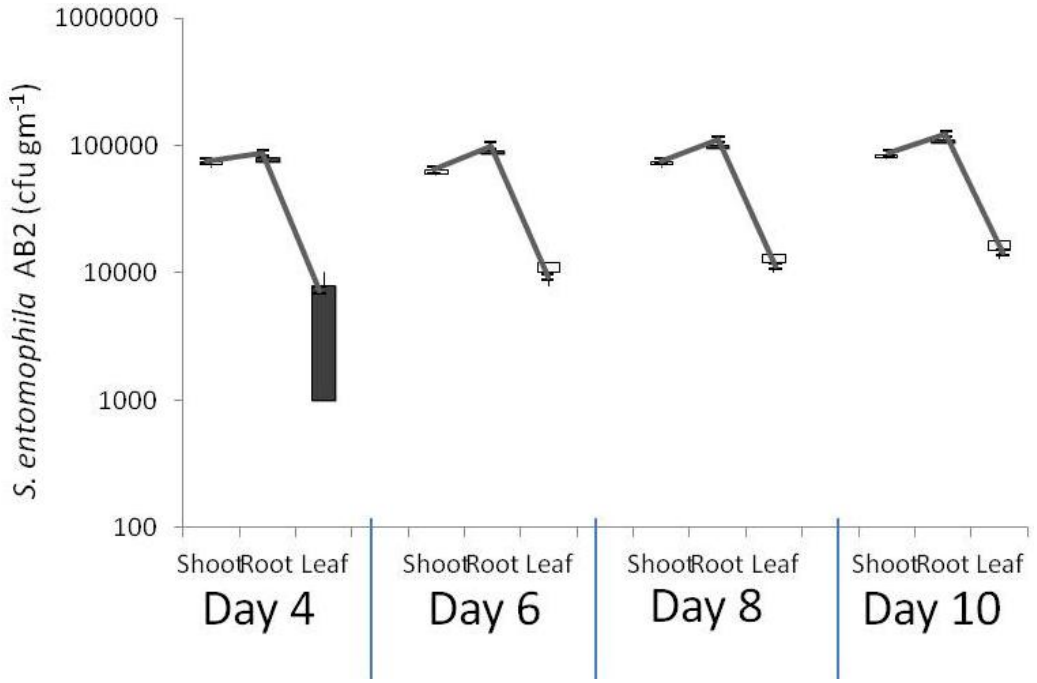

Figure 1. *S. entomophila* AB2 infestation through plant tissue from bacteria treated seed.

Systemic movement of *S. entomophila* AB2 through plant parts

When ground nut seeds were treated with *S. entomophila* AB2 and allowed to germinate, the bacterial strain was re-isolated from emerging shoots, roots as well as leaves, after surface sterilization. In box plot on the 10^{th} day, the highest and lowest population of the inoculum were recorded from root (11×10^4 cfu g^{-1}) and leaf (1.5×10^4 cfu g^{-1}) respectively. The direct cell count from *in vitro* experiment shows a slow but steady increase of inoculant population in all plant parts from the 4^{th} to 10^{th} day of the experiment but the count was more pronounced in leaf tissue (Figure 1).

The rhizobacterial strain *Serratia marcescens* 90 to 166 mediates induced systemic resistance (ISR) to fungal, bacterial, and viral pathogens (Press et al., 1997). Studies showed that systemic infestation of *S. marcescens* could effectively antagonise *Pyricularia oryzae* infestation in rice plant (Jaiganesh et al., 2007).

Conclusion

S. entomophila AB2, as a single biological agent for pest and disease management seems to be a lucractive alternative to chemical pesticides and fungicides in integrated crop management system. In the present study, it is understood that the seed application of *S. entomophila* AB2 could provide effective control against lepidopteran pest and fungal pathogens at the phyllosphere. It further assumes that the rhizospheric application would be reciprocated positively as an alternative to phyllospheric application because of systemic infestation of *S. entomophila* AB2 and colonization at different plant parts. This is the first report of systemic infestation of *S. entomophila* through plant tissue to impart protection against insect and fungal pests.

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REFERENCES

Akutsu K, Hirat M, Yamamota M, Hirayae K, Okuyama S, Hibi T (1993). Growth inhibition of Botrytis spp. by *Serratia marcescens* B2 isolated from tomato phylloplane. Ann. Rev. Phytopathol. Soc. Japan 59:18-25.

Amer M, Hussain SAS, Khan L, Khattak M, Shah GS (1999). The comparative efficacy of insecticides for the control of insect pest complex of cotton (*Gossypium hirsutum* L.). Pak. J. Biol. Sci. 2:1552-1555.

Chattopadhyay P, Chatterjee S, Gorthi S, Sen SK (2012). Exploring agricultural potentiality of *Serratia entomophila* AB_2: dual property of biopesticide and biofertilizer. British Biotechnol. J. 2:1-12.

Chattopadhyay P, Gorthi S, Chatterjee S, Sen SK (2011). Characterization of bacterial isolates as natural biocontrolling agent of bollworm from epizootic pest (*Heliothis armigera*). Pest Technol.

5:81-85.
Dennis C, Webster J (1971). Antagonistic properties of species groups of *Trichoderma* III Hyphal interaction. Trans. British Mycol. Soc. 57:363-369.
El-Tarabily KA, Soliman MH, Nassar AH, Al-Hassani HA, Sivasithamparam AH, Mckenna F, Hardy GEST (2000). Biological control of *Sclerotinia minor* using chitinolytic bacterium and actinomycetes. Phytopathology 49:573-590.
Glare TR, Corbett GE, Sadler AJ (1993). Association of a large plasmid with amber disease of the New Zealand grass grub, *Costelytra zealandica*, caused by *Serratia entomophila* and *Serratia proteamaculans*. J. Inver. Pathol. 62:165-170.
Grimont PAD, Jackson TA, Ageron E, Noonan MJ (1988). *Serratia entomophila* sp. nov. associated with amber disease in the New Zealand grass grub *Costelytra zealandica*. Int. J. Sys. Bacteriol. 38:1-6.
Hurst MR, Jones SM, Tan B, Jackson TA (2007). Induced expression of the *Serratia entomophila* Sep proteins shows activity towards the larvae of the New Zealand grass grub *Costelytra zealandica*. FEMS Microbiol. Letter 275:160-167.
Iyozumi H, Hirayae K, Komagata T, Tsuchiya K, Hibi T, Akutsu K (1996). Bio control of cyclamen grey mould by *Serratia marcescens* B2. Ann. Rev. Phytopathol. Soc. Japan. 62:559-565.
Jackson TA, Pearson JF, O'Callaghan M, Mahanty HK, Willocks M (1992). Pathogen to product - development of *Serratia entomophila* (Enterobacteriaceae) as a commercial biological control agent for the New Zealand grass grub (*Costelytra zealandica*). In: Jackson, T.A.; Glare T.R. (eds) Use of Pathogens in Scarab Pest Management. Intercept, Andover, UK. pp. 191-198.
Jaiganesh V, Eswaran A, Balabaskar P, Kannan C (2007). Antagonistic activity of *Serratia marcescens* against *Pyricularia oryzae*. Notule Botanicae Horti Agrobotanici Cluj 35:48-54.
Ji LY, Zhang WW, Yu D, Cao YR, Xu H (2012). Effect of heavy metal-solubilizing microorganisms on zinc and cadmium extractions from heavy metal contaminated soil with *Tricholoma lobynsis*. World J. Microbiol. Biotechnol. 28:293-301.
Kishore GK, Pande S, Podile AR (2005). Chitin supplemented foliar application of *Serratia marcescens* GP5 improves control of late leaf spot disease of Ground nut by activating defence related enzymes. J. Phytopathol. 153:168-173.
Nunez-Valdez ME, Calderon MA, Aranda E, Hernandez L, Ramirez-Gama RM, Lina L, Rodriguez-Segura Z, Gutierrez MC, Villalobos FJ (2008). Identification of a putative Mexican strain of *Serratia entomophila* pathogenic against root-damaging larvae of Scarabaeidae (Coleoptera). App. Environ. Microbiol. 74:802-810.
Nuñez-Valdez ME, Mahanty HK (1996). The *amb2* locus from *Serratia entomophila*confers anti-feeding effect on larvae of *Costelytra zealandica* (Coleoptera: Scarabaeidae). Gene 172:75-79.
O'Callaghan L, Mishra N, Meyerson A, Guha S, Motwani R (2002). Streaming-data algorithms for high-quality clustering. Proc. 18th Int. Conf. Data Eng. February 26-March 1, 2002, San Jose, CA, USA. pp:685-694.
Press CM, Wilson M, Tuzan S, Kloepper JW (1997). Salicylic acid production by *Serratia macrcescens* 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. Mol. Plant-Microb. Interact. 10:761.
Riveros AS, Giraldo CI, Gamboa A (2002). Microbiological control of black leaf streak disease. In: Mycosphaerella leaf spot disease of bananas: present status and outlook. Proc. 2nd Int. Workshop Mycosphaerella Leaf Spot Dis. May 20-23, 2002, San Jose, CA, USA. pp:287-296.
Someya N, Kataoka N, Komagata T, Hibi T, Akutsu K (2000). Biological control of cyclamen soil borne diseases by *Serratia marcescens* strain B2. Plant Dis. 84:334-340.
Vincent JM (1927). Distortion of fungal hyphae in presence of certain inhibitors. Nature 159:850.
Young SD, Townsend RJ, O'Callaghan M (2009). Bacterial entomopathogens improve cereal establishment in the presence of grass grub larvae. N.Z. Plant Prot. 62:1-6.