BIOLOGICAL SUPPRESSION OF SUGARCANE RED ROT BY *BACILLUS* spp. UNDER FIELD CONDITIONS

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SUMMARY

Yield of sugarcane (*Saccharum officinarum* L.) is critically limited by the red rot disease caused by *Colletotrichum falcatum* Went. Four native antagonistic bacteria *Bacillus subtilis* strains NH-100, *B. subtilis* NH-160, *Bacillus* sp. NH-217 and NH-69, that successfully suppressed the red rot disease under greenhouse conditions were evaluated to determine their efficacy as biological control agents in the field. Performance of the antagonistic strains was assessed on two different sugarcane varieties SPF-234 and Co-1148 under field conditions for three consecutive years. Two types of trials were conducted in which the red rot pathogen was inoculated by different methods to observe its direct suppression as well as the induction of systemic resistance. Three strains of the genus *Bacillus* reduced disease incidence by 45-49% in sugarcane plants challenged by pathogen inoculation in the stem and by 48-56% in the plants inoculated in the soil near the roots. The results of present study suggest a potential use of these strains in the development of commercial inoculants to be applied for the control of red rot disease.

Key words: sugarcane, red rot, *Bacillus*, *Colletotrichum falcatum,* biocontrol.

INTRODUCTION

Red rot of sugarcane (*Saccharum officinarum* L.), caused by the fungus *Colletotrichum falcatum* Went (perfect state: *Glomerella tucumanensis*), is a most disastrous disease affecting both yield and quality of the crop, and a serious threat to cane growers and sugar industry (Alexander and Viswanathan, 1996).

Control of this disease is based on the use of disease-free planting material, resistant varieties and fungicide treatments (Singh and Singh, 1989) which possesses many shortcomings, e.g. development of resistance against fungicides, emergence of new pathotypes and undesired effects on the environment (Mishra and Behera, 2009; Jayakumar *et al*., 2007).

An ecologically friendly alternative to these problems is biological control using rhizobacteria and their metabolic products (Vanitha *et al*., 2009; de Vasconcellos and Cardoso, 2009; Loper and Gross, 2007). Rhizobacteria inhibit plant pathogens by numerous mechanisms related to: (i) secretion of metabolites like siderophores, which suppress pathogens by sequestering iron (Matthijs *et al*., 2007); (ii) hydrolytic enzymes, which degrade the cell wall of many pathogens (El-Tarabily *et al*., 2006); (iii) antibiotics. which induce systemic resistance in plants.

Currently, only a limited number of biocontrol products are available on the market (Whipps, 1997) which makes it desirable to screen and evaluate more biocontrol bacteria. The development of an effective biological control of *C. falcatum* rests with the screening and evaluation of potential antagonistic bacteria capable of reducing red rot under field conditions. In fact, many rhizobacteria that antagonize pathogens *in vitro* failed to control them in the field (Reddy *et al.*, 1993).

Pseudomonads have successfully controlled red rot of sugarcane under field conditions as reported earlier (Viswanathan and Samiyappan, 2008; Hassan *et al.,* 2011) and in a previous study (Hassan *et al*., 2010a) *Bacillus* spp. reduced red rot incidence under greenhouse conditions but their potential to suppress this disease under field conditions was not determined. Antagonistic strains of the genus *Bacillus* are advantageous over other biocontrol agents in numerous ways, as they are ubiquitous in soils, sporulate excessively, have prolonged shelf life and enhance plant nutrition. Their efficacy in controlling many plant diseases has repeatedly been proven (see among others Siddiqui *et al*., 2005; Li *et al*., 2007; Gajbhiye *et al*., 2010).

In a previous study, we have isolated rhizobacteria antagonistic to *C. falcatum* and screened some strains capable of controlling red rot disease on sugarcane cv. SPF-234 under greenhouse conditions (Hassan *et al*., 2010a). The aim of this study was to evaluate the potential of the previously selected *Bacillus* strains to control red rot disease on two different sugarcane varieties SPF-234 and Co-1148 under field conditions.

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MATERIALS AND METHODS

Pathogen and biocontrol agents. A virulent strain of *C. falcatum* previously isolated from symptomatic sugarcane plants was used (Hassan *et al.*, 2010a). The strain was routinely grown *in planta* on stems of cv. SPF-234 to maintain its virulence and was also maintained on potato dextrose agar (PDA) *in vitro.*

Four bacterial antagonists, viz. *B. subtilis* strain NH-100 (EU627167), *B. subtilis* strain NH-160 (EU627169), *Bacillus* sp. strain NH-217 (EU627170) and NH-69 previously selected for their biocontrol activity towards *C. falcatum* (Hassan *et al.*, 2010a) and reference strain *Pseudomonas fluorescens* CHA0 with its proven biocontrol efficacy against sugarcane red rot (Viswanathan and Samiyappan, 2008; Hassan *et al.*, 2011) were used. Antagonistic strains were routinely cultured on Luria Bertani (LB) agar and maintained at -80ºC in LB broth with 20% glycerol for long-term storage.

Testing location. Efficacy of the antagonistic bacteria was explored in two different trials at the Shakarganj Sugar Research Institute of Jhang (Pakistan) for three consecutive years.

Cultivation of plants. Sugarcane cvs Co-1148 and SPF-234 were sown in a 20 × 40 m plot in February of each year. The plot had clay loam soil texture. The field was prepared by ploughing with chiesel once and twice with cultivator followed by planking. Double budded setts were placed end to end in furrows with R×R in cm. Plants were fertilized with N:P:K 150:100:100 kg ha^{-1} (Maqsood *et al.*, 2000). The full dose of phosphorus, potassium and 1/3 of nitrogen was applied at sowing time, while the remaining nitrogen was applied in two splits i.e. during the last week of March and the first week of May. The field was irrigated by flooding fortnightly throughout the crop growth.

Experimental design, treatments and bacterial inoculation. Treatments included inoculation of each antagonistic bacterium to the plant roots of the two sugarcane varieties grown either in pathogen-infested or pathogen-free soil. Inoculum of the antagonistic bacteria was prepared as described by Hassan *et al.* (2011). Briefly, the bacterial cells grown for 48 h on LB were suspended in 0.85% saline to a population density of 10^8 CFU ml^{-1}. About 5 ml of this inoculum was drenched twice manually in the soil near the roots of each plant as soil treatment in the 4th and 5th months after sowing (Hassan *et al.*, 2011). There were three replications for each treatment. Sugarcane plants inoculated with *P. fluorescence* CHA0 and 0.85% saline were used as positive and negative controls respectively. The experiment was arranged in a randomized complete block design.

Pathogen inoculation. The red rot pathogen was inoculated in two different ways in separate trials to evaluate the ability of potential antagonists to control the disease by either direct suppression or inducing systemic resistance in plants.

Challenge inoculation. Challenge inoculation of *C. falcatum* was done after six months of sowing and one month after the last bacterial application. The pathogen was inoculated as described (Srinivasan and Bhat, 1961; Hassan *et al.*, 2011), i.e. spores of *C. falcatum* grown on PDA for 6 days at 30±2°C were suspended in sterile distilled water (10^6 spores/ml) and 1 ml of the inoculum was injected with a syringe in a hole created on 3rd above ground node, which was then sealed with cotton and a cellophane membrane.

Soil inoculation. For soil inoculation (Viswanathan and Samiyappan, 2008; Hassan *et al.*, 2011) sugarcane stalks infested with *C. falcatum* were chopped and incorporated in the soil before sugarcane planting.

Disease assessment. Disease in each trial was assessed at two time periods (T_1 and T_2). Disease in plants inoculated with fungal spores (trial I) was assessed 7 and 8 months after sowing and 1 to 2 months after fungus inoculation. Canes were split opened at the 30th and 60th day after pathogen inoculation and symptoms like lesion width, top drying, transgression of lesions across the nodes and prominence of white spots in the stalks were observed. Disease intensity was scored on 0 to 9 scale as described by Srinivasan and Bhat (1961).

Disease incidence in the plants grown in contaminated soil containing red rot debris was assessed by counting the number of infected tillers at different intervals, i.e. 8 and 10 month after sowing. The disease incidence was calculated using the following formula.

Disease incidence = [Infected tillers/Total tillers] ×100

% disease suppression = 100-[(disease score/severity in treatment/disease score/severity in control) ×100]

Data analysis. Data were subjected to analysis of variance by standard procedures for a randomized complete block design using the Statistical computer software package (MSTAT-C).

RESULTS AND DISCUSSION

Development of chemical-free strategies of disease management based on biocontrol agents is an emerging area in crop protection. In the present study, some *Bacillus* strains significantly controlled red rot disease on sugarcane varieties Co-1148 and SPF-234 under field conditions. The antagonistic strains suppressed the pri-

mary infection (Soil inoculation) as well as secondary infection (Challenge inoculation) of red rot which is caused by certain vectors like the sugarcane borer (*Diatraea saccharalis*) and tools used in various cultural operations.

Efficacy of antagonistic strains against challenge inoculation of the pathogen. In the challenge inoculation experiment, all tested strains induced systemic resistance in sugarcane plants without direct contact with the pathogen. *B. subtilis* NH-100, *B. subtilis* NH-160 and *Bacillus* sp. NH-217 suppressed the disease by 45-49% with disease score of 4.3-4.6 (Table 1). Efficacy of these strains was statistically equal to that of reference strain *P. fluorescens* CHA0. Unidentified strain NH-69 also suppressed the disease up to 26% but its efficacy was low. Performance of the strains was equal on both sugarcane varieties and consistent throughout three years. The effect of varieties and year was statistically non significant (Table 1). Efficacy of strains comparative to each other was significant [F= 183.7, $df_{(strains, error)} = 5$, $p < 0.05$]. All the interactions were not significant except (strain × year) in both trials (Table 2).

In this experiment, bacterial strains were completely separated from the pathogen to elucidate the potential of biocontrol agents to induce systemic resistance which has already been reported (Choudary and Johri, 2009; Yang *et al*., 2011; Ramarathnam *et al*., 2011). These *Bacillus* strains also produced the broad-spectrum antifungal metabolite surfactin (Hassan *et al*., 2010b) which further characterize them as systemic resistance inducers since surfactin is a good inducer of systemic resistance in plants (Henry *et al*., 2011).

Efficacy of antagonistic strains against soil inoculation of the pathogen. In the soil inoculation experiment, all tested strains suppressed the disease effectively up to 56% with disease intensity 17.8 (Table 1). Performance of these strains was equal to that of reference strain *P. fluorescens* CHA0 (Table 1). The unidentified strain NH-69 was less efficient (19% disease suppression).

In this experiment, the effect of varieties and years was not significant, indicating the consistency in performance of the antagonists (Table 1). The effect of strains on both varieties was significant (F= 598.7 $df_{(strains, error)} = 5,144$ $p < 0.05$). All the interactions were not significant except (strain × year) in both trials (Table 2).

In soil inoculation, pathogen and bacterial strains were inoculated in soil, thus facilitating direct contact between fungus and bacteria. High performance of the strains may be due to their dual action i.e. induction of systemic resistance as well as direct suppression of the pathogen. This varying potential of antagonistic strains to suppress red rot disease supports the early findings

Table 1. Effect of *Bacillus* strains on red rot disease during three consecutive years.

	Challenge inoculation*		Soil inoculation**	
Strains	Disease intensity	% Disease suppression	Disease intensity	% Disease suppression
B. subtilis NH-100	4.3 A	49	17.8 A	56
B. subtilis NH-160	4.6 A	45	21.4 A	48
Bacillus sp NH-217	4.3 A	49	19.2 A	53
NH-69	6.2 B	26	33.2 B	19
P. fluorescence CHA0	3.6 A	57	18.2A	56
Control	8.4 C	0	41.1C	0
Year				
Year-1	5.4 D	36	26.1 D	37
Year-2	5.1 D	39	25.0 D	39
Year-3	5.2 D	39	24.4 D	41
Varieties				
Co-1148	5.1 E	39	25.9 E	37
SPF-234	5.3 E	37	24.5 E	40
Time period				
T1	4.6 F	45	23.5 F	43
T2	5.9 G	30	26.9 F	35

All the values are mean of three replicates and bearing same letter in the same column are not significant.

*T1: 30th day after fungal inoculation,T2: 60th day after fungal inoculation.

**T1: 8th month after sowing, T2: Harvesting time

*LSD $_{\text{strains, Years, varieties, Time period}}(0.05) = 1.269$ ($F_{strains}$=183.7 $df_{(strains, error)} = 5, 142$ $p < 0.001$)
(F_{var} =4.7 $df_{(var, error)} = 1, 142$ $p > 0.001$); ($F_{(TP)}$ =149, $df_{(TP, error)} = 1, 142$ $p < 0.001$); ($F_{(Year)}$ =4.2, $df_{(Year, error)} = 2, 142$ $p < 0.001$)

** LSD $_{\text{strains, Years, varieties, Time period}}(0.05) = 3.84$ ($F_{strains}$=598.7 $df_{(strains, error)} = 5, 144$ $p < 0.001$)
(F_{var} =19.6, $df_{(var, error)} = 1, 144$ $p > 0.001$); ($F_{(TP)}$ =109, $df_{(TP, error)} = 1, 144$ $p < 0.001$); ($F_{(Year)}$ =9.8, $df_{(Year, error)} = 2, 144$ $p < 0.001$)

Table 2. Analysis of variance interactions.

Source	Challenge Inoculation	Soil inoculation
Year × Variety	NS	NS
Year × Time	NS	NS
Variety × Time	NS	NS
Year × Variety × Time	NS	NS
Year × Strain	Significant	Significant
Variety × Strain	NS	NS
Year × Variety × Strain	NS	NS
Time × Strain	NS	NS
Year × Time × Strain	NS	NS
Variety × Time × Strain	NS	NS
Year × Variety × Time × Strain	NS	NS

NS = not significant

that biocontrol agents exert different mode of actions in disease control (Matthijs *et al*., 2007; El-Tarabily *et al*., 2006). In an earlier study, these *Bacillus* strains also showed a good ability to colonize the sugarcane roots and protect the cv. SPF-234 from red rot disease under greenhouse conditions (Hassan *et al*., 2010a).

Conclusively, ability of *Bacillus* strains to control red rot disease in both experiments and consistency in their performance under greenhouse and field conditions make them suitable candidates to be registered as biopesticide. However, further studies are required to develop their formulation and delivery system for effective disease management.

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