Comparative efficacy of potentially active antagonistic endophytic and rhizospheric bacteria against red rot of sugarcane

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Abstract: Sugarcane crop has attained the most significant position in the agrarian based economic country of India. The crop is source for the production of various valuable products which have become a part and parcel of daily needs. However, occurrence of disease especially; red rot disease is affecting the health status of crop. The disease is adversely affecting the economic status of crop. It not only affecting the growth and yield; but also, degrading the quality of juice. Thus, various biological strategies have been adopted for their effective management of red rot. As such, the present study dealt with comparing the *in vitro* efficacy of endophytic and rhizospheric bacteria isolated from sugarcane crop for suppressing the red rot pathogen. In the current study, total 51 bacteria were isolated from the sugarcane plant; 16 bacteria from the internal root tissues and 35 bacteria from the rhizospheric soil. Further, when the isolates were checked for *in vitro* antagonism against *C. falcatum*; it was found that a major proportion of endophytic bacteria isolated showed *in vitro* inhibition with above 50% inhibition when compare with rhizospheric bacteria. Therefore, it can be concluded that the *in vitro* biocontrol efficacy was higher in endophytic bacteria over rhizospheric ones.

Keyword: Rhizopsphere, Endophytes, Sugarcane, Red rot.

I. Introduction:

Sugarcane is an important agro-industrial cash crop cultivated in tropical and sub- tropical regions of the world. Globally, sugarcane is an important source for production of sugar and a raw material for sugar industry.Apart from, various other produce of sugarcane such as bagasse, molasses, filter wax, jaggery, khandsari and ethanol are being commercialized at large scale. Sugarcane is one of the most crucial crops of India where majority of population are dependent on it for their daily need. Various factors affecting the well-being of the crop are biotic and abiotic. Red rot is one of the threatening diseases widely affecting the sugarcane crop in the country. The fungal pathogen mainly responsible for such adverse condition is *Colletotrichum falcatum* which affects the well-being of standing crop. It causes severe loss in yield and quality of the susceptible cultivars in the Indian subcontinent (Satyavir, 2003; Duttamajumder, 2008). *C. falcatum* trigger severe loss of yield in many parts of sugarcane growing states in India. It can reduce cane weight by up to 29% and loss in sugar recovery by 31% (Hussnain and Afghan, 2006). The red rot pathogen hydrolysed the stored sucrose by producing the enzyme invertase which breaks the sucrose molecule into its components namely glucose and fructose. As a result, the quantity of molasses increases (Sehtiya, 1993).

Red rot disease of sugarcane is known to India in ancient times and measures to combat such disease are being taken since then. Therefore, continuous efforts are required for the effective improvement of this economical crop. A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people, and the environment. Rhizopheric bacteria are known for production of various stable secondary metabolites responsible for increasing the yield of crop as well as reduction of disease. Presently, another term "endophytic bacteria' is being used frequently as biocontrol agent isolated from inner side of crop plant. The endophytic bacteria are found to be closely associated with the plant and largely regarded as a untapped resource for the discovery of isolates with novel antifungal and plant growth-promoting traits (Lodewyckx et al., 2002; Rosenblueth et. al., 2006; Sturz et.al., 2008). For several crops, rhizospheric and endophytic bacteria have shown antagonistic effect against pathogen by providing beneficial effect on the health status of plant and inhibiting the pathogen growth. However, the main modes of action described are nitrogen fixation, production of phytohormones and antifungal compounds, and induced systemic resistance (Compant, 2005; Iniguez, 2004; Kuklinsky-Sobral, 2004). Biocontrol agents provide an excellent alternative to chemical pesticides and fertilizers. Thus, plant growth promoting rhizobacteria and endophytes are important, potentially active antagonistic agent capable of suppressing multiple pathogens and enhancing crop growth as well as yield. Consequently, sustaining and enhancing the growth and yield of sugarcane have become a major focus of research. Thus, present study aims to screen the potent antagonistic bacteria isolated from the rhizospheric and inner side tissue of root responsible for the *in vitro* suppression of plant diseases and to compare their efficacy in controlling the red rot fungus.

II. Methodology:

Sample collection:
Red rot susceptible healthy **s**ugarcane plant and the rhizospheric soil surrounding the sugarcane crop were collected in a sterile plastic cover and processed immediately for the isolation of bacteria.

Isolation of endophytic and rhizospheric bacteria:
Endophytic bacteria were isolated from the surface sterilized tissues of roots of sugarcane using the protocol of Viswanathan et.al., 2003 and Viswanathan and Samiyappan, 2001 on the Luria Bertani (LB) agar medium**.** Rhizospheric bacteria were also isolated from the soil sample of sugarcane by the serial dilution method on the LB agar media. The isolated endophytic and rhizospheric bacteria were then purified on the selected media of their isolation.

Dual culture Antagonism:
Isolated rhizospheric and endophytic bacteria were processed for dual-culture antagonism against red rot fungus i.e. *Colletotrichum falcatum* to assess their *in vitro* biocontrol efficiency (Viswanathan et. al., 2003). The dual culture antagonism was performed on Potato Dextrose agar (PDA) plates in which test fungal pathogen was placed in the centre of the agar plate and antagonistic bacteria (endophytic and rhizospheric) was line streaked at the corner of plate. All the plates were incubated for 7-10 days at 28ºC. After incubation, the percentage inhibition was calculated using the formula (C-T)/C x 100, where, C is the radial colony growth (mm) of fungal pathogen in control, and T is the radial colony growth (mm) in dual culture.

Characterization of efficient bacterial strain:
The selected potent antagonistic isolates were classified as gram positive or gram negative on the basis of Gram's staining. Further, isolates were biochemically characterized by performing various test such as catalase, oxidase, indole, H_2S production, citrate, methyl red, and voges-proskauer.

III. Results and Discussion:

Isolation of endophytic and rhizospheric bacteria:

A total of 51 bacteria were isolated from the rhizosphere soil and inside the root tissue of sugarcane plant (Table. 1) . However, the bacteria population isolated from the rhizospheric zone was greater in number with 35 bacteria (69%) and only 16 (31%) bacteria were isolated from inside the root tissue (Fig.1). Thus, it showed that rhizosphere is actually a factory of diverse bacterial population and can be isolated easily on a nutrient rich media. However, isolation of endophytes required more sensitive technique as compare to rhizosphere bacteria. As already mention in the methodology, endophytes can only be isolated from the surface sterilized process so as to kill the epiphytes microorganisms. This sterilization was achieved by the use of various chemical and disinfectant. However, during the process of isolation; some of the endophytic bacteria may get killed; if the sterilization process used for prolonged time. Therefore, the bacteria isolated from inside the root are less in number. Several studies have indicated that bacteria are the most numerous inhabitants of the rhizospheric zone. These are consistent with our study as number of bacteria obtained from the rhizosphere were higher in number.

Dual culture Antagonism:
Endophytic as well as rhizospheric bacteria act as a biocontrol agent which promote the growth of plants indirectly by protecting them from phytopathogens by means of inhibiting the growth of other organisms. Biocontrol test is detected by the observation of inhibition zone of the pathogenic fungus by the other organism. All the 51 isolates when checked for their antagonistic activity against the red rot fungus, it was found that out of the total 51 bacterial isolates, only 25 bacterial isolates showed *in vitro* antagonism against *Colletotrichum falcatum.*

Among the 16 endophytic bacteria, only 10 (RO-1, RO-2, RO-5, RO-6, RO-7, RO-8, RO-10, RO-13, RO-14 and RO-16) were showing *in vitro* antagonism against red rot fungus (Table. 2). Thus, 62% (10/16) of endophytes participated in the antagonism phenomenon. However, among the rhizospheric, out of 35 only 15 (SS-4, SS-5, SS-6, SS-7, SS-9, SS-11, SS-13, SS-15, SS-17, SS-18, SS-20, SS-21, SS-25, SS-27 and SS-30) i.e nearly 42% of the isolated rhizobacteria were showing inhibition against red rot fungus (Table.2). Percentage inhibition was measured accordingly. However, all the 10 endophytic bacteria were capable of showing *in vitro* antagonistic activity with above 50% inhibition (Fig. 2); and among rhizospheric bacteria only two (SS-4, SS-5) bacteria were showing above 60% inhibition of antagonism against pathogen; rest were showing antagonism in the range of 35% to 50% (Fig.3).

Bacteria residing in the rhizosphere or root-associated are generally termed as Plant growth-promoting rhizobacteria (PGPR) which can be defined as "a group of microorganisms that colonize the rhizosphere and roots of many plant species". They are able to provide nutrition and protection to the plant in one or several ways with the help of various mechanisms. Such beneficial effect is mainly due to the synthesis of phytohormone (indole acetic acid), avail the various elements to the plant such as phosphorus or iron with the mechanism of phosphorus solubilization and siderophore production respectively. Thus, PGPR confer beneficial effect on the plants which can ameliorate plant growth without the use of chemical fertilizer. Thus, rhizospheric bacteria can work as a substitute of chemical fertilizer and can be used as a promising biofertilizer in the agriculture field where sustainable approaches are required. *Pseudomonas*, *Bacillus*, *Azotobacter*, *Enterobacter*, and *Burkholderia* are some of the important class of PGPR microorganism which enhance plant growth and yield production (Glick, 1995). A major role of PGPR is through increasing the availability of nutrients in the rhizosphere region of the plant.

In the present scenario, endophytic bacteria have taken the advantage over rhizospheric bacteria. Endophytic bacteria can be defined as symptomless bacteria usually residing inside the plant tissue. They usually gain their entry from the rhizospheric zone and get intimately attached with the host plant. Thus, by establishing a close association with the host; they could establish mutualistic relationship and would not harm the plant (Hallmann *et al.*, 1997; Compant *et al.*, 2005).

The endophytes bacteria are more beneficial over rhizospheric bacteria as they are closely in the vicinity of plant and can affect the plant growth by providing more nutrition and suppress the pathogen growth by directly attacking on the pathogen. Also, endophytes have more efficacy as biocontrol agents as they reside in a closed environment; thus, avoiding the unnecessary circumstances faced by the bacteria to cope up with (Viswanathan et al., 2003). Also, studies have shown that endophytes have been able to control the disease of plants upto a remarkable extent. Besides, suppressing the pathogen growth, endophytes offers several other benefits over rhizospheric microorganisms (Rajkumar *et al.*, 2009) as PGPR microorganism provides to the plants. Biocontrol is actually achieved by the endophytic microbe by direct antagonism, and hyper parasitism mechanism. However, endophytes are also known to enhance plant growth and increase plant tolerance to certain biotic stresses by the secretion of certain secondary metabolites such as IAA production, phosphate solubilization and the production of a siderophores, HCN and ammonia production (Ryan *et al.*, 2008; Rajkumar *et al.*, 2009).

Morphological and Biochemical characterization of endophytes and rhizopsheric bacteria:

A total of 8 out of 10 endophytes were confirmed to be gram-positive organisms, and rest two were gram-negative organisms (Table 3). Similarly, the rhizobacterial isolates were showing dominance of gram-positive bacteria. Various biochemical tests were also performed as depicted in Table. 3. After visualizing the morphological and biochemical test; it was found that most of bacteria belonged to *Bacillus* group and only few of the bacteria belonged to *Pseudomonas* group. *Bacillus* and *Pseudomonas* group of bacteria have been previously been reported as endophytes from various agronomically important crop other than sugarcane. Viswanathan et al. 2003 also stated that *Bacillus* and *Pseudomonas* are able to colonize an ecological habitat in the host plant, thus, offering a potent biocontrol strategies against pathogen.

Table 1. Isolation of Bacteria from the rhizospheric soil and root portion of sugarcane

Place of isolation	No. of Isolates	Isolates
Root	16	RO-1, RO-2, RO-3, RO-4, RO-5, RO-6, RO-7, RO-8, RO-9, RO-10, RO-11, RO-12, RO-13, RO-14, RO-15, RO-16
Soil	35	SS-1, SS-2, SS-3, SS-4, SS-5, SS-6, SS-7, SS-8, SS-9, SS-10, SS-11, SS-12, SS-13, SS-14, SS-15, SS-16, SS-17, SS-18, SS-19, SS-20, SS-21, SS-22, SS-23, SS-24, SS-25, SS-26, SS-27, SS-28, SS-29, SS-30, SS-31, SS-31, SS-33, SS-34, SS-35

Table 2: Isolates showing percentage inhibition against *C. falcatum*

Isolates code	Inhibition of *C. falcatum*
Endophytic bacteria from root	
RO-1	51.42%
RO-2	50.93%
RO-5	55.75%
RO-6	68.56%
RO-7	62.41%
RO-8	66.05%
RO-10	59.84%
RO-13	54.99%
RO-14	63.25%
RO-16	60.16%
Rhizospheric bacteria from soil	
SS-4	68.92%
SS-5	62.52%
SS-6	44.52%
SS-7	48.81%
SS-9	46.42%
SS-11	49.99%
SS-13	40.36%
SS-15	45.77%
SS-17	35.94%
SS-18	43.23%
SS-20	46.71%
SS-21	47.56%
SS-25	46.78%
SS-27	47.80%
SS-30	46.34%

Table.3: Morphological and Biochemical characterization of antagonistic bacteria.

Strain	Gram's Staining	Motility	Catalase test	Indole test	Citrate utilization test	H_2S production test	MR test	VP test	Oxidase
RO-1	+	+	+	-	+	-		-	-
RO-2	+	+	+	-	-	-		-	-
RO-5	+	+	+	-	+	-		-	-
RO-6	+	+	+	-	+	-		-	-
RO-7	+	+	+	-	-	-		-	-
RO-8	-	+	+	-	+	-		-	+
RO-10	+	+	+	-	+	-		-	-
RO-13	-	+	+	-	-	-		-	+
RO-14	+	+	+	-	-	-		-	-
RO-16	+	+	+	-	-	-		-	-
SS-4	+	+	+	-	-	-		-	-
SS-5	+	+	+	-	+	-		-	-
SS-6	+	-	+	-	+	-		-	-
SS-7	+	-	+	-	+	-		-	-
SS-9	+	-	+	-	-	-		-	-
SS-11	+	-	+	-	+	-		-	-
SS-13	-	-	+	-	+	-		-	-
SS-15	-	+	+	-	-	-		-	-
SS-17	+	+	+	-	-	-		-	-
SS-18	-	+	+	-	-	-		-	-
SS-20	-	+	+	-	-	-		-	-
SS-21	+	+	+	-	+	-		-	-
SS-25	-	+	+	-	+	-		-	-
SS-27	-	+	+	-	+	-		-	-
SS-30	+	+	+	-	-	-		-	-

'+' represent Positive; '-' represent Negative

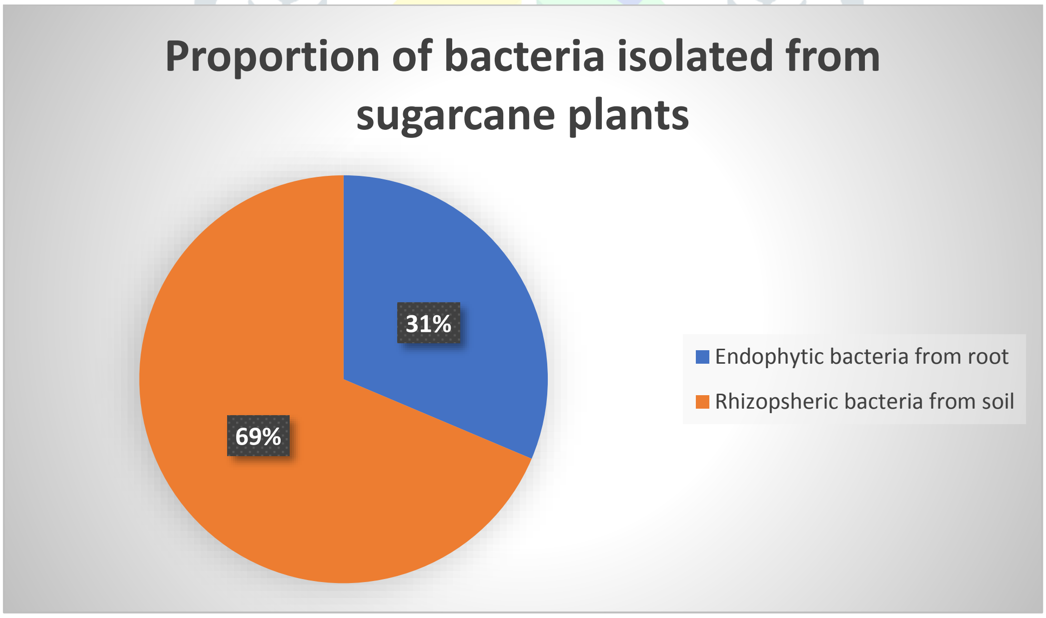

Figure.1 Graph comparing the proportion of bacteria isolated from rhizospheric zone and from the internal root system.

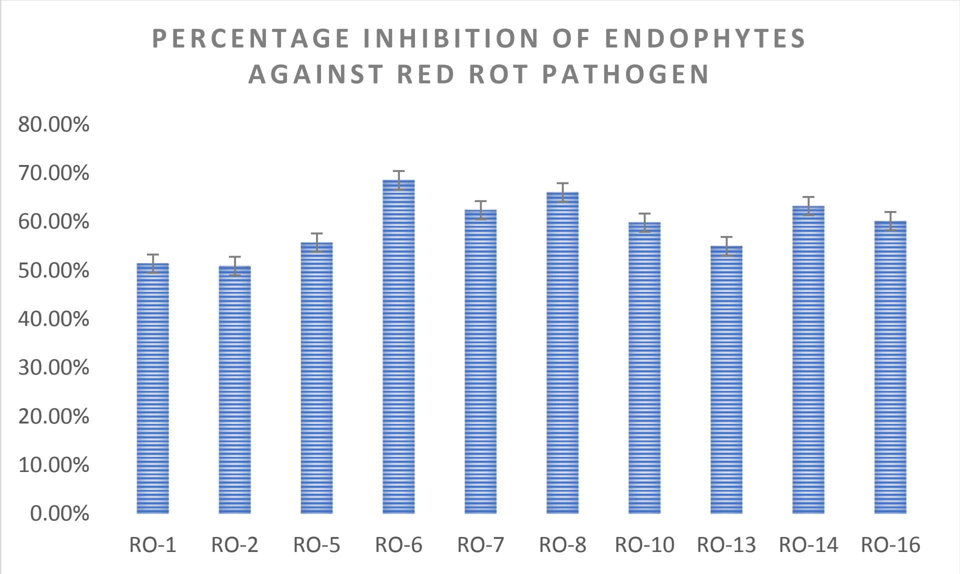

Figure.2 Graph showing *in vitro* antagonism of bacterial endophytes against *C. falcatum*

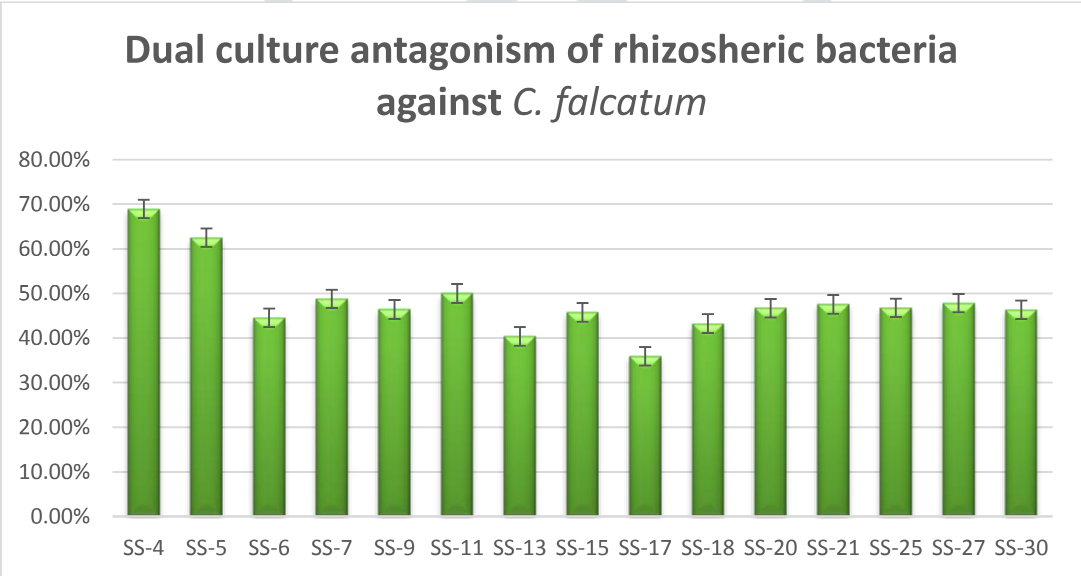

Figure. 3 Graph showing invitro antagonism of rhizobacterial against *C. falcatum*

IV. Conclusion:

The present study concluded that the bacteria isolated from root and rhizospheric soil were potentially active of showing inhibition against *C. falcatum*. However, after comparing the efficacy of endophytic and rhizospheric bacteria for their antagonism against red rot fungus; it was found that endophytic bacteria isolated was showing higher *in vitro* antagonism against red rot fungus over rhizospheric bacteria. Since, endophytic bacteria are found in close vicinity of host plant, thus, help in suppressing the red rot pathogen more efficiently. Thus, they can be used an important biocontrol agent in future.

References:

Compant S, Duffy B, Nowak J, Clement C, and Barka E A (2005): Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied Environment Microbiology*; 71:4951–4959.

Duttamajumder SK (2008): Red Rot of Sugarcane. Indian Institute of Sugarcane Research, Lucknow, India.

Glick, B R (1995). The enhancement of plant growth by free-living bacteria. *Canadian journal of microbiology*, *41*(2), 109-117.

Hallmann J, Quadt-Hallmann A, Mahaffee WF, and Kloepper JW (1997): Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* 43: 895-914.

Hussnain Z, and Afghan S (2006): Impact of Major Cane Diseases On Sugarcane Yield and Sugar Recovery. Annual Report, Shakarganj Sugar Research Institute, *Jhang, Pakistan.Infect.*; 5:535–544.

Iniguez A L, Dong Y, and Triplett E W (2004): Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. Mol. *Plant-Microbe Interaction*; 17:1078-1085.

Kuklinsky-Sobral J, Araujo W L, Mendes R, Geraldi I O, Pizzirani-Kleiner A A, and Azevedo J L (2004): Isolation and characterization of soybean associated bacteria and their potential for plant growth promotion. *Environmental Microbiology;* 6:1244–1251.

Lodewyckx C, Vangronsveld J, Porteous F, Moore E R B, Taghavi S, Mezgeay M, and der Lelie D (2002): Endophytic bacteria and their potential applications. *Critical Review Plant Science*.; 21:583–606.

Rajkumar M, Ae N, Freitas H (2009): Endophytic bacteria and their potential to enhance heavy metal phytoextraction. *Chemosphere* 77:153–160

Rosenblueth M, and Martı´nez-Romero E (2006): Bacterial endophytes and their interactions with hosts. *Mol. Plant-Microbe Interaction*.; 19:827–837.

Ryan, R P, Germaine K., Franks, A., Ryan, D. J., & Dowling, D. N. (2008). Bacterial endophytes: recent developments and applications. *FEMS microbiology letters*, *278*(1), 1-9.

Satyavir (2003): Red Rot of Sugarcane - Current Scenario. Indian Phytopathology;56: 245-254.

Sehtiya H L, Dhawan A K, Virk K S, and Dendsay J P S (1993): Carbohydrate metabolism in relation to Colletotrichum falcatum infection in resistant and susceptible sugarcane cualtivars. *Indian Phytopathology*.

Sturz A V, Christie B R, and Nowak J (2000): Bacterial endophytes potential role in developing sustainable systems of crop production. Crit. Rev. Plant Sci.; 19:1–30.

Viswanathan R, and Samiyappan R (2001): Role of chitinases in *Pseudomonas* spp. induced systemic resistance against *Colletotrichum falcatum* in sugarcane. *Indian Phytopathol*; 54 : 418-423.

Viswanathan, R, Rajitha R, Sundar RA, and Ramamoorthy V (2003): Isolation and Identification of Endophytic Bacterial Strains from Sugarcane Stalks and Their *In Vitro* Antagonism against the Red Rot Pathogen.*Sugartech*.Vol. 5 (l&2): 25 – 29