



Utilization of indigenous endophytic bacteria (*Bacillus spp.*) for their beneficial effect on growth of Sugarcane

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ARTICLE INFO	ABSTRACT
<p>Original Research Article Received on April 08, 2020 Revised on April 23, 2020 Accepted on May 02, 2020 Published on May 23, 2020</p> <p>Article Authors Beenu Shastri, Rajesh Kumar, Ram Ji Lal</p> <p>Corresponding Author Email beenu10shastri@gmail.com</p>	<p>Sugarcane (<i>Saccharum spp.</i> hybrid) occupies an important position among the various commercial crops of India. It is the second most important agro-industrial crop, next only to cotton in the country. Among the various approaches, use of microorganisms is one of the crucial approaches for enhancement of crop production. In recent years, endophytic bacteria are gaining much attention towards the sustainable management of the agricultural crops. They not only help in the plant growth but also in alleviating the various stresses due to their multi-faceted role. In current study, two endophytic bacteria <i>i.e.</i> <i>B. cereus</i> (strain S8) and <i>B. subtilis</i> (strain S 17) isolated from the sugarcane root and stalk were evaluated for their plant growth promotion activity <i>in vitro</i> and as well as <i>in vivo</i>. Data indicated that the percentage germination was higher in <i>B. cereus</i> (S8) over <i>B. subtilis</i> (S 17) treated sets when observed after 45 days of planting. Various growth parameters <i>viz.</i>, cane height, weight, number of shoots and internodes were also increased in the presence of endophytic bacterial inoculation over uninoculated control. Thus, it is concluded that sett treatment with indigenous endophytic bacteria (<i>Bacillus spp.</i>) can be exploited for enhancing cane yield and productivity in sugarcane culture.</p>
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Sugarcane is one of the most economically valuable agricultural crops grown worldwide and also main sources of sugar production. Across the world, it is cultivated on millions of hectare to produce thousand million metric tons crop. India is the second largest producer of sugarcane next to Brazil. It is one the most prestigious crop of Indian economy which not only helps in raising the livelihood for agriculture based farmers but also in sustaining the economy of the country. Various attempts have been made for improving the health status of this crop. Several researchers have reported the potential of plant growth promoting bacteria (PGPB) for integrated management of sugarcane

production (Chauhan *et al.*, 2012, Patel *et al.*, 2019). Currently, research is gaining attention towards using endophytic bacteria that can be easily defined as the group of endosymbiotic micro-organism that are colonized internally by the plants and can be easily isolated by various surface sterilization techniques (Mano and Morisaki, 2008). Microbial endophytes are known to be present in all the known plant species such as cotton, alfalfa, banana, pineapple, tomato, potato etc. (Afzal *et al.*, 2019). Endophytic bacteria are also known for their multi-dimensional role either by providing the host plant with nutrient or by protecting them against various biotic and a biotic stress.

The role of endophytic bacteria in the sugarcane crop is of utmost importance as sugarcane is vegetative propagated crop and once beneficial endophytic bacteria established inside they can be vegetative transmitted (Dong *et al.*, 1994, Viswanathan *et al.*, 2003). Various *Bacillus* spp. (*B. cereus*, *B. pumilus*, *B. subtilis*) have already been reported from the sugarcane crop (Mehnaz *et al.*, 2011). Since endophytes are reservoir of various metabolites that need to be further exploited for the crop benefits. Therefore, the overall aim of this study was to evaluate plant growth promoting endophytic bacteria *viz.*, *Bacillus cereus* and *B. subtilis* (that were isolated from internal tissues) under *in vitro* as well as *in vivo* condition for their beneficial effect on growth promotion of sugarcane.

Materials and Methods

Isolation of Endophytic Bacteria

The endophytic bacteria evaluated in the present study were isolated from the internal tissues (stalk and roots) of sugarcane as reported by (Shastri *et al.*, 2020). Based on their potent plant growth promoting (PGP) ability, *B. cereus* (strain S8) and *B. subtilis* (strain S17) were used for further studies.

In Vitro Evaluation of Plant Growth Promoting Activity

The selected isolates of *B. cereus* (S8) and *B. subtilis* (S17) were qualitatively tested for various plant growth promoting abilities *viz.*, indole acetic acid production (IAA), phosphate solubilisation, siderophore production, ammonia production and ACC deaminase etc., under *in vitro* condition as per the procedure of Shastri *et al.* (2020).

In Vivo Effect of Endophytic Bacterial Inoculation on Growth Parameters

The bacterial endophytes based on their PGP characteristics were further utilized for their growth promoting effect under pot experiment. The bacterial suspension was prepared as per the method described by (Hassan *et al.* 2010, 2011) with slight modification. The sugarcane setts of variety Co 1148 were pre-soaked in the prepared bacterial suspension (10^{-6} spores/ml) for an hour and five two-budded setts were planted/pot (30x46cm) filled with sterile soil in the month of February.

The same bacterial suspension was also applied in the soil after 4th and 5th months of planting. There were three treatments *viz.*, (i) *B. cereus* strain S8 (ii) *B. subtilis* strain S17 and (iii) Control (without bacterial inoculation). The experiment was laid in a randomized pattern with eight replicates. After planting, various plant growth parameters were recorded time to time. The percentage germination was recorded at 45th days after planting (DAP) and calculated with the following formula:

$$\text{Percentage germination} = \left(\frac{\text{No. of bud germinated}}{\text{No. of bud sown}} \right) \times 100$$

Cane height, girth, weight, number of shoots and internodes were also recorded at the time of harvest of the crop.

Results and Discussion

The *in vitro* as well as *in vivo* effect of endophytic bacteria (*Bacillus* spp.) on plant promoting activity is summarized here under.

In vitro Evaluation of Plant Growth Promoting Properties

The selected endophytic bacteria isolated from the root (*B. cereus*; strain S8) and stalk (*B. subtilis*; strain S17) showed positive effect on growth promotion ability. Six different PGP traits were evaluated and in both the strains out of six, five were present in *B. cereus* (S8) and only four were present in S17 strain of *B. subtilis* (fig 1). It was observed that both the isolates were found positive for phosphate solubilization, ammonia and siderophore production. However, IAA and ACC deaminase activity was produced by *B. cereus* (S8) only and absent in *B. subtilis* (S17). The HCN was produced by *B. subtilis* (S17) and absent in *B. cereus* (S8).

In Vivo Effect of Endophytic Bacterial Inoculation on Growth Promotion Activity

After visualizing their plant growth abilities under *in vitro* condition, both the above *Bacillus* spp. strains were also evaluated for their plant growth promotion activity under *in situ* condition. Data revealed that percentage germination was significantly higher in both the bacterial inoculated treatments (S8 and S17 strains) when compared with the uninoculated control.

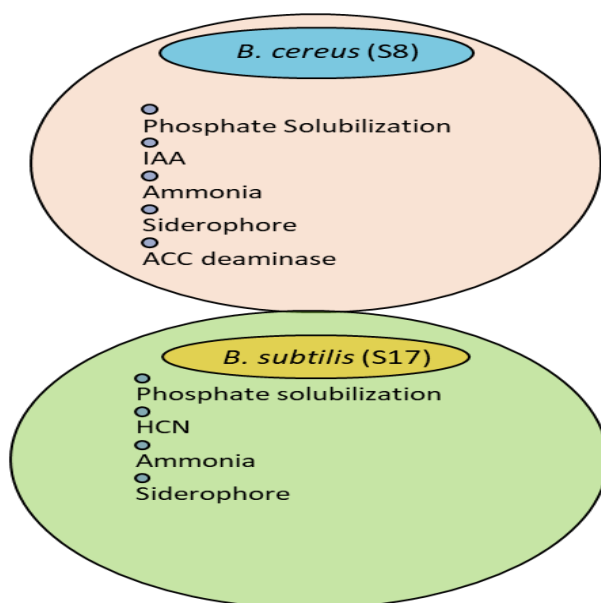


Fig 1. Plant growth promoting activity in *B. cereus* (S8) and *B. subtilis* (S17)

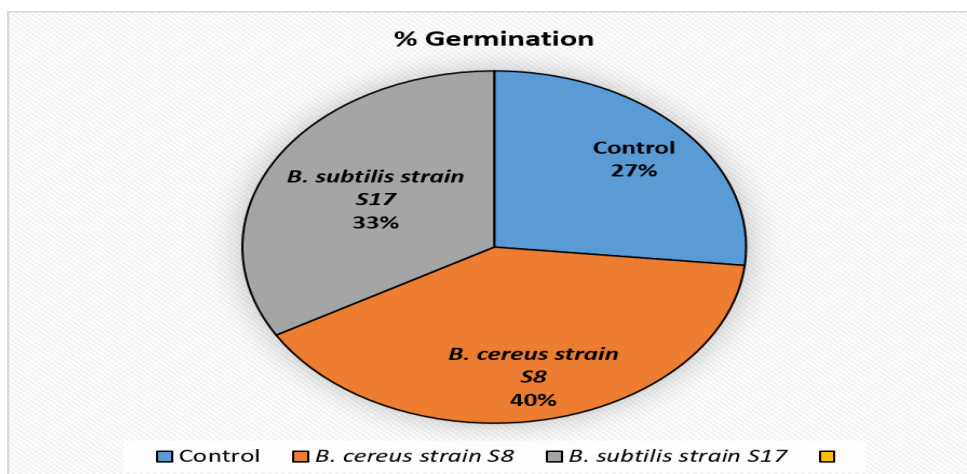


Fig 2. Percentage germination in plant inoculated with *B. cereus* (S8) and *B. subtilis* (S 17) over uninoculated control

Table 1. Effect of inoculation of endophytic bacterial on various growth parameters

Treatment	Cane Height (cm)	Number of Shoot per Treatment	Cane Weight (kgs)	Cane Girth (cm)	Number of Internode
Uninoculated Control	160	9	0.5	2.2	22
<i>B. cereus</i> (S8)	184	11	0.7	2.3	25
<i>B. subtilis</i> (S17)	170	15	0.6	2.2	24

However, after 45 days of planting of sett, percentage germination was higher in *B. cereus* strain S8 (40%) followed by *B. subtilis* strain S17 (33%) over uninoculated control (27%), respectively (fig 2). The reason attributed for the higher germination in *B. cereus* (S8) was mainly due to the presence of various plant growth promoting (PGP) traits together. It was further observed that endophytic bacterial inoculated plants showed

enhancement in various growth parameters viz., cane height, girth, weight, number of shoots and internodes when compared with the uninoculated control (table 1). It has been well documented in the literature that the endophytic bacteria can promote the plant growth by secreting various metabolites that leads to enhancement in plant growth. The cane height was highest in *B. cereus* (S8) inoculated plants (184 cm) than *B. subtilis* (S 17) i.e. (170 cm).

The increase in cane height in case of *B. cereus* (S8) treated setts might be due to the secretion of IAA, an additional PGP traits which *B. subtilis* (S 17) does not possess. However, in both the cases, height was more than uninoculated control (160 cm). Similarly, total number of shoots/treatment were higher in *B. subtilis* (S17) inoculated plants than *B. cereus* (S8) treatment. The cane girth was also higher in both the bacterially inoculated plants (S8 and S 17) over uninoculated control. However, there was no significant difference in the cane girth among the treatments. Single cane weight was also found to be significantly higher in *B. cereus* (S8) inoculated plants than *B. subtilis* (S 17). Thus, pot experiment demonstrated that the bacterially inoculated sugarcane plants were superior in terms of plant height, shoot counts and various other growth parameters.

The study conducted by Chauhan *et al.* (2012) also congruent with the current study. Various researchers (Quecine *et al.* 2012, Egamberdieva *et al.* 2017) also investigated the plant growth promotion by endophytic bacteria under field experiment. The overall conclusion drawn from their studies was that bacterially inoculated plant showed more beneficial effect on the growth parameter of plants over the control plant. It is concluded from the present study that both the strains of endophytic bacteria (*B. cereus* and *B. subtilis*) can be exploited in sugarcane culture for enhancing cane yield and productivity.

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