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Biological Control and Plant-Growth Promotion by *Bacillus* Strains from Milk

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Abstract Six-hundred bacterial strains from human milk and milk from *Sahiwal* cows, *Holstein Friesian* cows, and buffaloes were screened for their ability to suppress phytopathogenic fungi under *in vitro* conditions. A consortium of 3 strains, viz., *Bacillus lentimorbus* B-30486 (B-30486), *B. subtilis* B-30487 (B-30487), and *B. lentimorbus* B-30488 (B-30488), isolated from *Sahiwal* cow milk resulted in better biological control and plant-growth promotion than single-strain treatments. For commercial-scale production of a bioinoculant, the solid-state fermentation of sugarcane agro-industrial residues, i.e., molasses, press mud, and spent wash, using the consortium of B-30486, B-30487, and B-30488, resulted in a value-added product, useful for enhancing plant growth. The application of the consortium to sugarcane fields infested with *Fusarium moniliforme* and *Colletotrichum falcatum* resulted in a reduction of mortality and significantly higher (P=0.05) plant height, number of tillers, and cane girth when compared with the control. Furthermore, under field conditions, the treatment of sugarcane with the consortium resulted in significantly (P=0.05) greater plant growth compared with nonbacterized plants. Accordingly, this is the first report on the effective use of bacteria isolated from milk for biological control and enhancing plant growth under field conditions. Furthormore, a solid-state fermentation technology was developed that facilitates the economic utilization of agro-industrial residues for environmental conservation and improving plant and soil health.

Key words: Plant-growth promotion, biological control, cow's milk, *Bacillus*, sugarcane, solid-state fermentation, press mud, spent wash

Microbes already provide many economically valuable products and processes that are used every day. However, with the advent of modern biotechnology tools and processes, the potential applications of microbes are expected to increase significantly. Consequently, this has prompted microbiologists to continue to search for novel useful microbes from sources that remain uncharacterized [16]. The current authors unexpectedly observed that the application of cow's milk to plant seedlings enhanced their overall growth. Thus, experiments were undertaken to investigate the significance of these observations. Interestingly, an ancient *Sanskrit* text on the science of plant life describes that milk can be used to change the color of flowers and enhance the taste of fruit [25]. The effectiveness of cow's milk against the powdery mildew (*Sphaerotheca fulginea*) that afflicts zucchini squash (*Cucurbita pepo*) has already been demonstrated under greenhouse conditions [2]. A lot of work has also been done on psychrotropic bacteria, due to their importance in milk and dairy products [19]. However, no microbiological studies have yet been conducted on plant-growth-promoting bacteria from milk. Therefore, the present study focused on the bacteria found in cow teats and/or udder that enter and mix with aseptically drawn milk, with the objective to look for novel microbes from a hitherto uncharacterized ecological niche.

Public concern over the continued use of agrochemicals that damage human health and the environment continues to grow. Increasing productivity to meet the food demands of a growing population also means that available resources and waste need to be recycled. Therefore, these concerns are driving the search for more environmentally friendly methods to promote plant growth that will contribute to the goal of sustainability in agriculture. In 1999–2000, India was the second largest producer of sugarcane (*Saccharum officinarum* L.), next to Brazil, with a production of 300MT. Australia, South Africa, Cuba, China, and tropical and subtropical countries are also major contributors to the world production of sugarcane [5]. In India, a number of large-scale distilleries operate in conjunction with sugar mills, and the waste products include press mud (mud and dirt residues after clarifying the sugarcane juice), molasses

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(final residue from sugar crystallization), and spentwash (waste water generated from the distillation of the alcohol fermenter wash) [34]. These wastes have never been fully exploited, because of the lack of viable technology for economic recycling. However, microbial biotechnology may play a role in providing processes and products for sugarcane agro-industrial residues.

The present authors have been studying plant-growth-promoting rhizobacteria (PGPR) as potential plant-growth promoting and biological control agents, and reported that PGPR strains with promising biological control activity can be selected based on combining their inhibiting zones, abiotic stress tolerance, and root-colonizing capacity [13–18, 29]. Previous research on the microbiology of milk has focused on psychrotropic bacteria, due to their importance in milk and dairy products [19]. However, no plant-growth-promoting bacteria have yet been isolated from milk. Therefore, this study reports on three plant-growth promoting strains of *Bacillus*, viz., *B. lentimorbus* B-30486, *B. subtilis* B-30487, and *B. lentimorbus* B-30488, which were isolated from the milk of *Sahiwal* cows. These isolates, used individually or as a novel mixed consortium, provide a unique synergism that can control phytopathogenic fungi and promote plant growth under field conditions. It is also shown that this consortium of strains is a good candidate for the solid-state fermentation of sugarcane agro-industrial residues, where the product can be utilized as both an organic fertilizer and a microbial pesticide.

MATERIALS AND METHODS

Milk Samples and Bacterial Strains

Human milk was collected from mothers with 6-12-week-old breast-fed infants. Milk from pure bred *Sahiwal* cows was collected from the Gajaria farm, Department of Animal Husbandry, Government of Uttar Pradesh, Lucknow. Milk from *Holstein Friesian* cows was collected from the Indian Military Farm, Central Command Headquarters, Indian Army, Lucknow. Samples of buffalo milk were taken from a local commercial dairy farm. All the milk samples were collected in sterile containers under sanitary conditions from cleaned (sanitized) teats. The milk samples were drawn midstream in the morning from the teats directly into clean sterile glass vessels free from phosphate residues, then stored in an icebox while being transported to the laboratory. Serial dilutions of the samples were plated on a Nutrient Agar (from HI-MEDIA Laboratories Pvt. Ltd., Bombay, India) (NA), as described earlier [14]. Fifty of the predominant morphologically distinct colonies of bacteria that appeared on the plates were selected from each sample of healthy human, *Sahiwal* cow, *Holstein Friesian* cow, and buffalo milk, where three individual milk samples were taken in each case, resulting in a total of 600 selected colonies that were then purified by subculturing each individual strain on an NA plate to obtain a pure culture for further screening. Each isolate was stored in an aqueous solution of 30% glycerol at –25°C.

Screening of Bacterial Isolates for Their Ability to Inhibit Phytopathogenic Fungi and Promote Plant Growth

The 600 bacterial isolates were screened under *in vitro* conditions, as described earlier [15], for their ability to inhibit the growth of phytopathogenic fungi, viz., *Fusarium moniliforme*, *Colletotrichum falcatum*, *Sclerotium rolfsii*, *Alternaria solani*, *Penicillium* sp., *Pythium aphanidermatum*, *Phytophthora palmivora*, *Curvularia lunata*, *Sclerotinia sclerotiorum*, and *Aspergillus niger*. Samples from 4 different bacterial colonies on the NA plates were streaked around the edge of a 90-mm diameter Petri plate, and the plates incubated at 28°C for two days. An agar plug inoculum of the fungi to be tested (5-mm square) was then transferred to the center of the Petri plate from a source plate of the fungi. After incubation for 5 to 7 days, inhibition zones were readily observed in the case of the bacterial strains with biological control activity, as the fungal growth around the streak was inhibited. In the case of the bacterial strains without biocontrol activity, there was no inhibition of fungal growth around the streak, and the fungi grew towards the edge of the plate. The strains that showed an inhibition zone of at least 2 mm were designated as positive and used for further work. Of the 600 isolates tested *in vitro* using this criterion, only 150 were found to possess biocontrol activity.

Nonsterile field soil from the National Botanical Research Institute farm at Lucknow was used to evaluate the plant-growth promotion potential of the 600 isolates in a greenhouse. Bacterial inocula were prepared for maize (*Zea mays* L.) seeds and evaluated in the greenhouse using four different sets of 16 maize seedlings for nonbacterized (control) and bacterized seeds, respectively, as previously described [15]. Trays (35×35 cm) with 16 (4×4) spaces per tray (spaces were 7 cm wide, 10 cm deep, and 1 cm apart from each other) were used to grow the maize. Each space was filled up to 8 cm with the nonsterilized soil, and tap water (25 ml) added to provide 20% moisture before planting the seeds. Four bacterized seeds were planted in each space. The experiment in the greenhouse was carried out using four different sets of 16 maize seedlings for the control and bacterized seeds, respectively. In each set, the dry weights of the 21-day-old seedlings were recorded. For the bacterial strain to be ranked as a plant-growth promoter, the seedlings treated with the bacteria were required to have a dry weight that was at least 10% greater than that of the control plants. Among the 150 bacterial isolates tested in the greenhouse using this criterion, 50 were found to be plant-growth promoting.

The 50 bacterial isolates identified to suppress pathogenic fungi *in vitro* and enhance plant growth were then further screened for their abiotic stress tolerance. The tolerance of the strains towards salt (NaCl), pH, and temperature was tested by growing them on a nutrient broth (NB; beef extract 5.0 g, peptone 10.0 g, sodium chloride 5.0 g, distilled water 1,000 ml, pH 7.2) under various stress conditions, including 6% salt (NaCl), 5–9 pH, and 55°C temperature. The viable cells were counted by removing samples at various times in the presence or absence of stress, as indicated. First, the 50 strains were subjected to 6% salt stress. Fifteen out of the 50 isolates were able to grow overnight (14–16 h) in the presence of 6% salt stress at 28°C in a New Brunswick Scientific (U.S.A.), Innova Model 4230 refrigerated incubator shaker at 180 rpm. The 15 isolates found to be tolerant to 6% salt stress were also able to grow overnight at pH 9.0. However, only 3 of the 15 isolates were able to grow overnight at 55°C. Serial dilutions of each sample were spotted (25 µl) onto NA plates and incubated at 28°C in triplicate, as described earlier [17]. The viable cells were then counted after 2–3 days.

The 3 bacterial isolates were biochemically characterized, as described earlier [9], in conjunction with a fatty acid analysis performed by CABI Bioscience (Egham, Surrey, U.K.). As a result, the isolates were identified as *B. lentimorbus* B-30486 (B-30486), *B. subtilis* B-30487 (B-30487), and *B. lentimorbus* B-30488 (B-30488) and have been deposited under the Budapest treaty in the ARS Patent culture collection, United States Department of Agriculture, Illinois, U.S.A.

Fungal Growth Inhibition

The interaction of *F. moniliforme* and *C. falcatum* with B-30486, B-30487, and B-30488, individually and in a consortium, was elucidated in a liquid medium using a dual culture test based on growing the bacterium/consortium on a nutrient broth in the presence or absence of the fungi. Agar disks (5-mm in diameter) of *F. moniliforme* and *C. falcatum* were individually inoculated in 150-ml Erlenmeyer flasks, each containing 50 ml of a nutrient broth. Suspensions of B-30486, B-30487, and B-30488, individually and in a consortium, containing 1×10^9 CFU/ml were inoculated and the flasks incubated at 28°C for 7 days in an incubator shaker under static conditions; the control cultures were grown without bacteria. The mycelial dry weights of the fungus grown in the presence and absence of B-30486, B-30487, and B-30488, individually and in a consortium, were determined by filtering out the spent media using a Whatman filter paper no. 1 and drying the fungal mass on the filter paper at 60°C for 3 days.

Microscopic observation of the inhibition of *F. moniliforme* by B-30486 was made using a light microscope (Nikon Eclipse E-400, Japan), and the microbial interactions between *F. moniliforme* and B-30486 in the 30-day-old sugarcane seedling roots were monitored by scanning electron microscopy (SEM) using a Phillips XL-20 (Holland) scanning electron microscope, as described earlier [17].

Compatibility Between B-30486, B-30487, and B-30488

To examine their compatibility, B-30486, B-30487 and B-30488 were individually tested for *in vitro* antibiosis against each other. The tests were conducted on 90-mm NA plates. Cultures of B-30486, B-30487, and B-30488 were individually grown in NB and incubated for 3 days at 28°C in a New Brunswick Innova refrigerated incubator shaker at 180 rpm. To test the antibiosis between B-30486, B-30487, and B-30488, 100 µl of a 1×10^6 CFU/ml suspension of one bacterium was spread over the agar surface and dried. Using a sterile cork borer (4 mm diameter), 4 equidistant holes were bored in the 4 corners of each plate. For the first set of experiments, holes 1, 2, 3, and 4 were filled with 100 µl of a 1×10^8 CFU/ml suspension of B-30486, B-30487, B-30488, and rifampicin (50 µg/ml), respectively. Thus, each plate contained two challenged bacteria, one negative control bacterium (one spread over the agar surface), and rifampicin as the positive control. Another set of experiments was also conducted, where each hole was filled with 100 µl of a 72-h-growth spent medium, obtained after centrifuging out the bacterial cells and passing the medium through a 0.45 µ membrane filter (Millipore Corporation, Bedford, MA, U.S.A.). The plates were incubated at 28°C for 24 h and examined for the emergence of clear zones, indicating inhibition, or a lack of clear zones, indicating compatibility between the strains. Each combination of bacteria was replicated at least three times. Since B-30486, B-30487, and B-30488 did not exhibit any antibiosis against each other, the strains were considered to be compatible. Thus, cultures of B-30486, B-30487, and B-30488 were individually grown in NB and incubated for 2 days at 28°C. Thereafter, a consortium of the 3 bacteria was prepared by mixing the B-30486, B-30487, and B-30488 cultures at approximately 1×10^9 CFU/ml, based on a ratio of 1:1:1 (v/v).

Use of Sugar Factory Sulfitation Press Mud and Distillery Spent Wash as Carrier

For the purpose of commercialization, the sugar factory sulfitation press mud and distillery spent wash produced at Dhampur Sugar Mills Ltd., Dhampur, India were utilized as a carrier and fermented with a consortium of B-30486, B-30487, and B-30488. Cultures of B-30486, B-30487, and B-30488 were grown in 2-l flasks containing 1.5 l of molasses diluted with water based on a ratio of 1:5 and incubated for 3 days at 28°C in an Innova refrigerated incubator shaker at 180 rpm. Thereafter, a consortium of the 3 bacterial strains was obtained by mixing the 3 cultures at approximately 1×10^9 CFU/ml, based on a ratio

of 1:1:1. The consortium was then diluted with water based on a ratio of 1:10, resulting in approximately 1×10^9 CFU/ml. About 300 tons of fresh sulfinated press mud, obtained as a byproduct of the clarification of sugarcane juice with lime and sulfur dioxide, was laid out on a cement floor with windrows that were 150 meters long, 2.5 meters wide, and 1.5 meters high. The press mud was churned and homogenized either manually or by means of an aero tiller before adding about 600 l of the bacterial consortium, i.e., 2 l of the consortium/ton of press mud, followed by re-mixing. Within 2–3 days, the temperature of the windrows increased to 70–75°C, whereupon the windrows were churned twice a day and the spent wash sprayed daily for up to 40 days to maintain 55–65% moisture. Thereafter, the spraying was stopped and the windrows turned regularly for 3–5 days to reduce the moisture in the fermented product to about 30%. After about 45 days, the temperature of the windrows was seen to fall to 40–45°C, at which point the product was totally fermented and ready for packaging or application.

Pot Trials

Two-hundred-and-seventy earthen pots (12" diameter) were filled with field soil and sterilized for 1 h at 15 lb/in^2 on three successive days. Different bacterial formulations and consortia were mixed with the upper 5 cm of the pot soil at a rate of about 20 gm/pot. Next, spores from a 10-day-old culture of *F. moniliforme* were mixed in sterilized distilled water to make a final solution with a spore count of 10^6 spores/ml, then 135 setts of sugarcane were dipped in this solution and one sett planted in each pot. Likewise, a solution of *C. falcatum* was also prepared with a final spore count of 10^6 spores/ml, then 135 setts of sugarcane were dipped in this solution for 30 min prior to planting one sett in each pot. All the pots were kept in a glasshouse for 90 days at 25±2°C. For each treatment, a set of 9 pots was used as one replicate, and three replicates used for data recording. The per cent germination and red rot and wilt disease incidence were all recorded.

Field Trials

Field trials using sugarcane were conducted to evaluate the plant-growth promotion potential of the consortium. Sugarcane setts, variety Co 89003, were directly planted in the prepared carrier of fermented sugar factory sulfitation press mud and distillery spent wash containing the consortium. The sugarcane trial was carried out in May 2001 at Dhampur Sugar Mills Ltd., Dhampur and Dhampur Sugar Mills Ltd., Rozagaon, Faizabad. After 12 months of plant growth, the number of tillers, plant height, girth of the sugarcane, millable cane, and cane yield were recorded in May 2002. The field experiments were conducted using a randomized block design with three replicates. The yield per plot was recorded at the time of harvest and calculated as described earlier [28]. Statistical analysis of the data was performed using the Data Analysis tool of Microsoft Excel (Microsoft Office XP version), according to the method described by Panse and Sukhatme [20]. The differences were considered significant at P=0.05.

RESULTS AND DISCUSSION

Selection of Strains

The present study screened bacteria for their ability to control phytopathogenic fungi, promote plant growth, and be tolerant of abiotic stress. To this end, 600 bacterial isolates from healthy human, cow (*Sahiwal* and *Holstein Friesian*), and buffalo milk were screened for their ability to inhibit the growth of plant pathogenic fungi, viz., *F. moniliforme*, *C. falcatum*, *S. rolfsii*, *A. solani*, *Penicillium* sp., *P. aphanidermatum*, *P. palmivora*, *C. lunata*, *S. sclerotiorum*, and *A. niger* under *in vitro* conditions. The highest percentage of bacterial strains showing biological control activity against phytopathogenic fungi was found in the milk from the *Sahiwal* cows, followed by the human milk and the milk from the *Holstein* cows and buffaloes (Table 1). As a result, 150 isolates showing an inhibition zone of at least 2 mm were selected as positive, and screened further in greenhouse trials based on growing bacterized maize seeds in nonsterile soil and comparing the bacterized maize with control maize plants grown without bacterial treatment. The treatment of the maize seeds with the 50 bacterial isolates resulted in a higher dry weight by at least 10% when compared with the control plants. Meanwhile, the abiotic stress tolerance of the 50 strains towards salt (NaCl), pH, and temperature was tested based on their ability to tolerate various abiotic stress conditions, including 6% salt, 5–9 pH, and 55°C temperature. First, the 50 isolates were subjected to 6%

Table 1. Screening of bacterial strains under *in vitro* conditions for ability to suppress pathogenic fungi.

Pathogenic fungi	% of biological control bacteria[a]			
	Human	*Sahiwal* cow	*Holstein* cow	Buffalo
Fusarium moniliforme	2	21	5	9
Colletotrichum falcatum	0	17	0	8
Sclerotium rolfsii	0	8	0	0
Alternaria solani	8	8	8	0
Penicillium sp.	8	0	0	0
Pythium aphanidermatum	8	8	8	0
Phytophthora palmivora	8	8	8	0
Curvularia lunata	17	8	8	0
Sclerotinia sclerotiorum	0	17	8	0
Aspergillus niger	0	17	8	0

[a]Values are means of 6 replicates.

salt stress, and 15 out of the 50 isolates were able to grow overnight (14–16 h) in the presence of 6% salt stress at 30°C. These same 15 isolates were also able to grow overnight at pH 9.0. However, only 3 out of the 15 isolates, all isolated from the milk of *Sahiwal* cows, were able to grow overnight at 55°C. Therefore, the three strains, B-30486, B-30487, and B-30488, were selected as having the ability to control phytopathogenic fungi, promote plant growth, and be tolerant to abiotic stress. Strains of *B. amyloliquefaciens* and *B. subtilis* have already been shown to synthesize plant-growth promoting substances, such as gibberellins and indole acetic acid [30], extracellular phytase [10], chitinase [32], and antifungal peptides [22]. Seed inoculants containing strains of *B. subtilis* and related species have also been shown to increase yields of various crops [3, 7, 24, 33].

Interaction of B-30486, B-30487, and NRRL B-30488 with *F. moniliforme* and *C. falcatum*

The interaction of B-30486, B-30487, and NRRL B-30488 with *F. moniliforme* and *C. falcatum* was studied under *in vitro* conditions. When *F. moniliforme* and *C. falcatum* were grown in a nutrient broth for 7 days, the dry weight of the fungal mass in the presence of B-30486R, B-30487, and B-30488, individually and in a consortium, was inhibited within a range of 47–70%, compared with the control (Table 2). The percentage inhibition of *F. moniliforme* and *C. falcatum* by B-30486R, B-30487, and B-30488 varied when the strains were used individually, yet was maximal in the presence of the bacterial consortium (Table 2). A time-course investigation of the interaction between *F. moniliforme* and B-30486 was also conducted, and light microscopic examination of mycelial samples of *F. moniliforme* collected after its interaction with B-30486 for 2 h revealed some degradation in the fungal hyphae. When the interaction with B-30486 was prolonged to 6 h, many *F. moniliforme* hyphae were seen to have lost their cytoplasmic content (data not shown). The light microscopy observations were also confirmed by scanning electron microscopy, where the fungal hyphae of *F. moniliforme* incubated with B-30486 showed alteration and distortion of the hyphal cell wall (Fig. 1). Four hours after inoculating the fresh roots of 30-day-old sugarcane seedlings, B-30486 and *F. moniliforme* were detected on the root surface (Fig. 1A). The proliferation of B-30486 cells on the hyphae of *F. moniliforme* and degradation of the hyphae were clearly noticeable by 16 h. Twenty-four hours after inoculating the seedlings, the B-30486 cells lysed the *F. moniliforme* (Fig. 1B).

Table 2. Interaction of B-30486R, B-30487, and B-30488, individually and as a consortium, under *in vitro* conditions against sugarcane phytopathogenic fungi *F. moniliforme* and *C. falcatum*.

Treatment	Fungal dry mass (mg)[a]
Fusarium moniliforme (FM)	
1. FM	164.8±7.1
2. FM+B-30486	74.7±2.2
3. FM+B-30487	86.5±3.9
4. FM+B-30488	75.7±3.0
5. FM+consortium	49.4±2.4
Colletotrichum falcatum (CF)	
1. CF	95.4±4.4
2. CF+B-30486	44.9±2.7
3. CF+B-30487	57.4±2.2
4. CF+B-30488	53.7±2.7
5. CF+consortium	30.3±1.5

[a]Values are means of 4 replicates±SE.

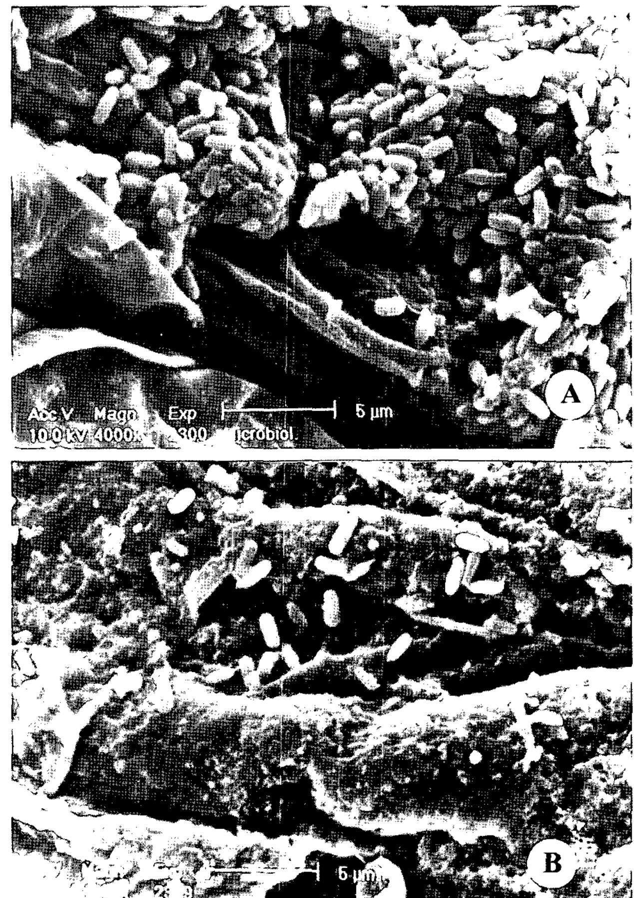

Fig. 1. Scanning electron micrographs of *Fusarium moniliforme* samples collected 4 h (A) and 24 h (B) after interaction with *Bacillus lentimorbus* B-30486. Bar=5 µm.

The *in vitro* inhibition of the growth of *F. moniliforme* and *C. falcatum* by B-30486 when co-inoculated in liquid medium (Table 2), along with the light microscopic observations, would seem to indicate that cell wall hydrolytic enzymes are produced by B-30486. Further evidence of the lysis of the fungal cell wall by B-30486 was observed in a SEM photograph (Fig. 1). The production of extracellular enzymes by biocontrol bacteria is a well-documented

Table 3. Effect of B-30486R, B-30487, and B-30488, individually and as a consortium, on disease incidence of *F. moniliforme* and *C. falcatum* in sugarcane under pot conditions.

Treatment[a]	Percent germination	Disease incidence	Percent disease control
Fusarium moniliforme			
1. FM	61.3±4.67	39.4±1.67	0.0
2. FM+B-30486	81.9±3.37	19.3±2.17	51.0
3. FM+B-30487	80.7±5.79	18.2±3.33	53.8
4. FM+B-30488	83.6±4.33	16.5±4.37	58.1
5. FM+consortium	95.7±2.91	7.6±2.49	80.7
Colletotrichum falcatum			
1. CF	63.2±4.17	36.2±1.79	0.0
2. CF+B-30486	84.7±6.67	18.6±2.33	49.3
3. CF+B-30487	83.2±4.79	15.4±1.69	58.0
4. CF+B-30488	87.6±2.79	11.2±1.59	69.5
5. CF+consortium	96.6±3.67	4.9±0.79	86.6

[a]For each treatment, a set of 9 pots was selected as one replicate. After 3 months of plant growth, the calculation of the percent germination and *F. moniliforme* and *C. falcatum* disease incidence was performed using data recording the mean of 3 replicates ±SE for each treatment used.

phenomenon that is thought to be involved in the lysis of the cell wall of phytopathogenic fungi [8, 12, 17, 21]. Among *Bacillus* species, *B. subtilis* and occasionally *B. megaterium*, *B. cereus*, *B. pumilus*, and *B. polymyxa* have already been studied as biocontrol agents. *B. subtilis* produces several kinds of antibiotic, e.g., bacillomycin, iturin, mycosubtilin, bacilysin, fengymycin, and mycobacillin [27]. The current results support the hypothesis that these hydrolytic enzymes contribute to biological control efficacy. The present observations also indicate that the production of antimicrobial metabolites and lytic enzymes could be involved in the ability of B-30486 to inhibit the growth of *F. moniliforme*. Furthermore, the finding that a consortium of B-30486, B-30487, and B-30488 increased the *in vitro* inhibition of *F. moniliforme* and *C. falcatum* (Table 2) and provided better biological control and plant-growth promotion (Table 3) than the individual strains is in agreement with previous reports [6, 8, 21]. For example, it has already been reported that mixtures of PGPR strains, in either two-way or three-way combinations, give greater protection against cucumber angular leaf spot disease caused by *P. syringae* pv. *lachrymans* under field conditions [23] than single-strain treatments. This may be because mixtures of several strains can produce a more stable rhizosphere community, provide several mechanisms of biological control, and suppress a broader range of pathogens [11, 16, 21]. Thus, compatible mixtures of certain biological control strains with antagonism as the main mechanism of action have allowed greater disease suppression than the use of individual control strains [6, 11].

Greenhouse Studies

The plant-growth promotion potential of B-30486, B-30487, and B-30488, individually and in a consortium, was tested in a greenhouse using pots. The bacterized sugarcane plants exhibited better germination than the control. When the setts were treated with the individual bacterial strains and *F. moniliforme*, the percent germination ranged from 80–83%, whereas it was 95% with the bacterial consortium. The wilt disease incidence was also lowest (7%) with the bacterial consortium, along with the *C. falcatum* disease incidence. The percent germination for the pots treated with either the individual bacterial strains (83–87%) or the consortium (96%) was higher than that for control (63%).

Among the bacterized plants, the consortium produced the best results in terms of better seedling growth and survival. Therefore, the consortium, rather than a single strain, was used for further detailed work. So far, Gram-negative bacteria, especially *Pseudomonas* strains, have been extensively investigated as bioinoculants [31], whereas Gram-positive bacteria, like *Bacillus* spp., have received less attention, as Gram-positive organisms are less tractable for genetic study [1, 7]. Nonetheless, the efficacy of Gram-positive bacteria such as *Bacillus* spp., is striking. One notable feature of *Bacillus* spp. is the formation of endospores, which are resilient structures capable of surviving desiccation, heat, oxidizing agents, and UV and γ irradiation [26]. As such, these qualities confer exceptional ecological advantages to bacilli and are conducive to long-term storage and relatively easy commercialization of *Bacillus*-based products [4].

Field Studies

The treatment of sugarcane with the consortium resulted in a lower mortality and significantly greater (P=0.05) plant height, number of tillers, and cane girth when compared with the control (Table 4), represented by a 30% and 80% improved mortality, 12% and 6% increase in plant height, 106% and 43% increase in the number of tillers, and 39% and 34% increase in the cane girth in the fields infested with the sugarcane pathogens *F. moniliforme* and

Table 4. Effect of consortium on biological control of wilt and red rot fungi and plant-growth promotion in sugarcane (var. Co 95255).

Observation	Treatment[a]		CD at 5%
	Control	Bacterized	
Fusarium moniliforme			
Mortality (%)[b]	30	0	
Plant height (cm)[c]	161.8±3.21	181.4±1.79	22.17
Number of tillers/plant[d]	3.1±0.33	6.4±0.69	3.73
Girth of cane (cm)[e]	5.8±1.21	8.1±1.67	0.34
Colletotrichum falcatum			
Mortality (%)[b]	80	0	
Plant height (cm)[c]	152±1.91	162±2.46	11.1
Number of tillers/plant[d]	4.6±0.67	6.6±0.79	2.26
Girth of cane (cm)[e]	5.8±1.37	7.8±1.33	2.26

[a]Bacterized with consortium consisting of B-30486, B-30487, and B-30488 (1×10^9 CFU/ml).
[b]Values are means of 6 replicates ±SE, based on the seedling survival among 50 seedlings after 3 months of plant growth.
[c]Mean shoot lengths (cm) of 6 replicates ±SE, based on 20 plants after 12 months of plant growth.
[d]Mean number of tillers/plant for 6 replicates ±SE, based on 20 plants after 12 months of plant growth.
[e]Mean girth of 6 replicates ±SE, based on 20 plants after 12 months of plant growth.

Table 5. Effect of consortium on plant-growth promotion of sugarcane (var. Co 89003) in Dhampur and Rozagaon.

Observation	Treatment[a]		CD at 5%
	Control	Bacterized	
Dhampur			
Number of tillers/plant[b]	6.0±0.69	8.4±1.23	2.71
Plant height (cm)[c]	220±2.67	251±3.17	35.06
Girth of cane (cm)[d]	7.2±1.33	9.4±1.79	2.49
Millable cane[e]	4±0.33	9±0.90	5.66
Cane yield/plot (t/ha)[f]	67.69±1.65	85.31±2.33	19.93
Rozagaon			
Number of tillers/plant[b]	6.2±0.67	11.0±0.91	5.43
Plant height (cm)[c]	200±2.47	246±3.11	52.06
Girth of cane (cm)[d]	9.1±1.49	11.24±1.69	2.42
Millable cane[e]	5±0.91	8±0.46	3.39
Cane yield/plot (t/ha)[f]	64.48±2.39	82.82±3.71	20.74

[a]Bacterized with consortium consisting of B-30486, B-30487, and B-30488 (1×10^9 CFU/ml).
[b]Mean number of tillers/plant for 6 replicates ±SE, based on 20 plants after 12 months of plant growth.
[c]Mean shoot lengths (cm) of 6 replicates ±SE, based on 20 plants after 12 months of plant growth.
[d]Mean girth of 6 replicates ±SE, based on 20 plants after 12 months of plant growth.
[e]Mean number of millable canes for 6 replicates ±SE, based on 20 plants after 12 months of plant growth.
[f]Mean cane yield/plot of 6 replicates ±SE, at the time of harvesting after 12 months of plant growth.

C. falcatum, respectively (Table 4). The plant-growth promotion ability of the consortium was also evident based on the significantly higher (P=0.05) number of tillers, plant height, cane girth, millable cane, and cane yield for the bacterized sugarcane compared with the control, represented by a 40% and 77% increase in the number of tillers, 14% and 23% increase in the plant height, 30% and 23% increase in the cane girth, 125 and 60% increase in the millable cane, and 26% and 28% in the cane yield at Dhampur and Rozagaon, respectively (Table 5). An overall increase in the sugarcane plant growth and profuse rooting were also observed in the inoculated plants compared with the uninoculated controls (Fig. 2). Numerous bacteria have already been shown to directly stimulate the growth of plants using varied mechanisms [8], including improving the mineral and water uptake, changing the hormonal balance of plants, and generally improving the entire root system [8, 17, 18].

Fig. 2. Effect of consortium on plant-growth promotion of sugarcane.
Upper panel [left to right]: portion of field-grown bacterized sugarcane compared with control (non-inoculated). *Lower panel* [left to right]: sugarcane plants showing profuse rooting in inoculated plants compared with control.

In conclusion, B-30486, B-30487, and B-30488 isolated from cow's milk were shown to have the ability of biological control and plant-growth enhancement under field conditions. Furthermore, the treatment of the major waste byproducts from the sugarcane industry with the bacterial consortium resulted in a material that functions as both an organic fertilizer and a microbial pesticide. Thus, an appropriate solid-state fermentation technology was developed that can enable economic utilization of agro-industrial residues. Furthermore, in view of the bulk availability of such residues, they can serve as an ideal substrate for the microbial production of value-added products. Accordingly, the recycling of agro-industrial residues, such as the major waste byproducts of the sugarcane industry, can be a wise approach to the gainful and productive use of available resources for conserving the environment and improving plant and soil health.

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