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Antagonistic potential of phylloplane *Bacillus subtilis* PBs4 isolate against grain mold fungi of sorghum in India

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Abstract— *In India grain mold disease incited by a number of saprophytic fungi is a major disease of sorghum that results into qualitative as well as quantitative loss of produce due to infection of maturing grains. Use of chemical fungicides on maturing grains may impose serious health hazards, therefore, use of antagonistic microbes may provide an effective alternative to contain this serious problem. In vitro assessment of Bacillus subtilis isolates collected from phylloplane of sorghum, rice, cotton, soybean, pigeonpea and chilli was done by dual culture method for their potential antagonism against major grain mold fungal pathogens of sorghum i.e. Fusarium moniliforme, Alternaria alternata, Curvularia lunata and Phoma sorghina. Amongst the six Bacillus isolates, maximum inhibition of mycelial growth was registered by PBs4 against F. moniliforme (75.99%), A. alternata (81.86%), C. lunata (78.09%) and P. sorghina (67.68%) followed by PBs3 and PBs2 indicating maximum antagonistic potential of Bacillus subtilis PBs4 against sorghum grain mold causing fungi.*

Keywords— ***Alternaria alternata, Bacillus subtilis, Curvularia lunata, Fusarium moniliforme, Phoma sorghina, Grain Mold***

I. INTRODUCTION

Bacillus species have been widely recruited in biocontrol of plant diseases for more than 50 years because of their well-developed secretary system that produce structurally diverse secondary metabolites displaying a wide spectrum of antagonistic activity (Araujo *et al*., 2005; Awais *et al.*, 2007; Liu *et al*. 2007). Phylloplane isolates of *Bacillus subtilis* are particularly useful as biocontrol agents as they increase yields and stimulate plant growth and are also responsible for increasing total biomass production, leaf area, chlorophyll concentration and nitrate reductase activity. *Bacillus subtilis* has been reported as the most promising biocontrol agent under a variety of environmental conditions against various plant pathogens (Chen *et al*. 2008). As a largest source of wide array of bioactive natural compounds *Bacillus* species are reported to be used as an antifungal antagonist (Abd El-Ghafar, 2008; Shrivastava *et al*., 2013; Narasimhan & Shivakumar, 2014). *Bacillus subtilis* showed resistance only to penicillin, amoxicillin and ampicillin antibiotics. The antibiotics obtained from the culture of certain members of the *B. subtilis* family produce a wide variety of antimicrobial substances like chitinase, sublancin, subtilin, subtilosin A, and TasA those are ribosomal antibiotics and others, like bacillaene, bacilysin, chlorotetain, difficidin, mycobacillin, rhizocticins and lipopeptides including fengycins, iturins and surfactins are produced under the facilitation of non-ribosomal peptide synthetases and polyketide syntheses (Araujo *et al*., 2005; Awais *et al.*, 2007; Akond *et al*. 2016, Zhen *et al*. 2023). Several members of the genus *Bacillus*, including *Brevibacillus* and *Paenibacillus* spp. are capable of producing more than 70 different antibiotics (Araujo *et al*., 2005; Awais *et al.*, 2007). Many airborne, seed-borne and soil borne diseases of rice, wheat, sugarcane, jute, groundnut, cotton, rubber, soybean, tobacco and vegetables etc. are reported to be

controlled by *B. subtilis* (Araujo *et al.*, 2005; Yogo *et al*., 2011; Xiao *et al*., 2014; Smitha *et al.*, 2017). Akond *et al.* (2016) reported that the members of *Bacillus* genus can colonize the root and leaf system of plants and compete thereby suppressing the growth of plant pathogens. Phylloplane microorganisms like *B. subtilis* are receiving considerable attention as potential prophylactic bioagents to protect the plants against foliar fungal pathogens. Due to its capacity and potential to colonize on phylloplane the *B. subtilis* acts as a barrier restricting invasion of pathogens. As documented by Hoch *et al.* (1993) amongst bacterial biocontrol agents, *Bacillus* genus possess a large genetic biodiversity encompassing diverse climatic conditions ranging from soil to sea water and even the extreme environmental situations of hot springs. Therefore, this bacterium retains several valuable traits and is one of the major sources of potential biopesticides of microbial origin (Ongena and Jacques 2008). US Food and Drug Administration (USFDA) has granted the "Generally Regarded as Safe" (GRAS) status to *B. subtilis* which is thus recognized as non-pathogenic (Harwood and Wipat 1996) that is essential for its application as a biopesticide. Further, *Bacilli* are known to produce spores (Piggot and Hilbert 2004) those are extremely resistant dormant forms capable of withstanding unfavourable pH, lack of nutrients, lack of water, high temperatures, etc. Spores are produced by the bacteria under unfavourable environmental conditions to help them survive through adversities.This study was aimed to compare six isolates of *B. subtilis* obtained from phylloplane of different field crops of Central India, for their ability to suppress grain mold fungal pathogens of sorghum through the production of cyclic lipopeptides, hydrolytic enzymes and siderophores.

II. METHOD

Isolation of grain mold causing fungi and pathogenicity test

Diseased sorghum ear heads were collected from different districts of Vidarbha region of Maharashtra, India for isolation of grain mold causing fungi,. Four species of fungi i.e. *Fusarium moniliforme, Alternaria alternata, Cuvrularia lunata* and *Phoma sorghina* were isolated on Potato Dextrose Agar (PDA) medium by tissue isolation technique. Pathogenicity tests were performed for collected fungal cultures. For further study, one isolate of each specie with proven pathogenicity was maintained as axenic culture on PDA media (Petkar, 2021).

Isolation of *B. subtilis* from phylloplane

By employing serial dilution technique 6 isolates of *B. subtilis* were collected from phylloplane of healthy leaves of six field crops i.e. sorghum, rice, cotton, soybean, pegionpea and chilli on Nutrient Agar (NA) medium. For preparing water blank test tubes with 9 ml distilled water were sterilized in an autoclave. To remove dust particles and loosely adhering unassociated microbes, after gentle rinsing in the sterilized distilled water for a few minutes the collected leaf samples were placed in 100 ml sterilized distilled water and shaken vigorously for 30 min. Serial dilutions were prepared from 10^{-1} to 10^{-10} dilutions. One millilitre sample from 10^{-7} dilution was transferred on NA media in Petri-plates. Inoculated Petri-plates were incubated for 48 hours at room temperature. One representative growth of visibly distinguishable bacterial colony was transferred to a fresh NA media plate to develop the pure culture and designated as PBs1 to PBs6, respectively, corresponding to the field crops mentioned earlier (Petkar, 2021).

Biochemical properties of phylloplane *B. subtilis* isolates

Biochemical tests viz., acid production from carbohydrates, catalase test, gas production from carbohydrates, gelatin liquefaction, Gram's reaction, H_2S Production, KOH test, starch hydrolysis, methyl red test, etc. were performed for biochemical confirmation of *B. subtilis* isolates. All the isolates of *B. subtilis* were further evaluated for plant growth promotion properties viz., IAA production and phosphate solubilization etc.

Gram's reaction

Bacillus subtilis was identified by Gram's staining and by studying the morphological characters of the bacteria (Jha *et al.* 2016 & Kapali *et al.* 2016). Gram's staining was performed using crystal violet as main stain (30 seconds), potassium iodide/ Lugol's iodine solution (30 seconds) as fixer, 95% alcohol as decolourizer and saffranin (10 seconds) as counterstain. After staining drop of cedar wood oil was placed on the slide and smear was examined under the oil immersion lense.

Potassium hydroxide (KOH) solubility test

Two drops of KOH were placed on a glass slide. A colony of *B. subtilis* isolate culture was picked up from the medium with the help of inoculation needle and mixed with KOH for 10 seconds and needle was raised for 0.5 to 2 cm to form thread which was treated as positive test (Petkar, 2021).

Starch hydrolysis

Starch is a complex carbohydrate (polysaccharide) composed of two constituents; amylose, a straight chain polymer of 200-300 glucose units and amylopectin, a larger branched polymer with phosphate groups. The positive test indicates by the presence of amylase enzyme,

an exoenzyme that hydrolyses (cleaves) starch, into maltose (disaccharide) and some monosaccharides such as glucose. For this test *B. subtilis* isolate was inoculated on starch agar plates and incubated for 7 days. After incubation, the plates were flooded with Lugol's iodine solution. Appearance of clear zone indicated complete hydrolysis of starch and reddish zone indicated partial hydrolyses of starch to dextrin. (Xiao Hua Zhang *et al*. 2014)

Catalase test

During aerobic respiration microorganisms (MO's) produce hydrogen peroxide (H_2O_2) which is lethal to themselves. In some MO's catalase enzyme is present that breaks down H_2O_2 to water (H_2O) and oxygen (O_2) and helps them to survive under aerobic conditions. Catalase test was performed by adding H_2O_2 to Trypticase Soy Agar slant culture of *B. subtilis* isolate. Release of free oxygen gas (O_2) bubbles indicated positive catalase test **(**Mandla *et al*. 2017**).**

Gelatin liquefaction

Gelatin is a protein produced by hydrolysis of collagen which is a major component of tendons and connective tissue in humans and other animals. Bacterium capable of producing proteolytic exoenzyme gelatinase, hydrolyzes gelatin to amino acids resulting into liquefaction. To conduct this test *B. subtilis* isolates were inoculated to stab of a nutrient gelatin (i.e. Nutrient broth + 1.5% gelatin) and incubated for 7 days and observed for liquefaction. Uninoculated tubes were compared as control. Liquified tubes showed presence of gelatinase activity i.e. positive test for gelatin hydrolysis and tubes that remained solid indicated negative test for gelatin hydrolysis (Avsar *et al.* 2017).

Hydrogen Sulphide (H_2S) production

Production and liberation of H_2S gas results due to the activity of bacterium on sulphur containing amino acids. H_2S gas reacts with lead acetate to turn it black. So, lead acetate paper was prepared by moistening the filter paper in saturated solution of lead acetate to test the H_2S production. In the tubes containing peptone water (i.e.peptone 1%, cystine 0.01%, NaCl 0.5%) inoculated with *B. subtilis* isolate, the lead acetate paper was kept holding by the plugs above the culture, without touching the media. The tubes were incubated for 3 days. Turning of lead acetate paper strips black indicated positive test for H_2S production (Abbo *et al.* 2014).

Indole production

Tryptophan is an essential amino acid that is oxidised by some bacteria capable of producing tryptophanase enzyme resulting into formation of indole, pyruvic acid and ammonia. The indole test was performed by inoculating bacterium into 1% tryptone broth (i.e. 10 gm of tryptone in one litre of distilled water), and production of indole during the reaction was detected by adding Kovac's reagent (i.e. P-di-methylamino benzaladehyde 50g, amyl alcohol 750ml, HCl 250ml) that produces a cherry-red reagent layer. In this procedure medium was distributed in test tubes and autoclaved. *B. subtilis* isolate was inoculated and incubated for 48hrs and Kovac's reagent (1ml) was added in incubated test tubes. The tubes were allowed to stand to permit the reagent to surface on top. Development of a cherry (deep) red colour on the top layer of the tube indicated positive test for indole production. Absence of red colouration indicated negative test for indole production (Zhang *et al*. 2008).

Acid and Gas production from carbohydrates

Dextrose broth (Nutrient broth + 0.5% dextrose) was prepared with the test reagent (20 ml broth + 0.2 ml Bromo-Cresol purple). In each test tube 10 ml dextrose broth was filled and one durham tube was placed in inverted position. After sterilization of test tube it was inoculated with *B. subtilis* isolates and incubated for 3 days at room temperature. When colour of indicator changed from blue to yellow it indicated formation of acid and gas by it's accumulation in Durham tube that was a positive test whereas in uninoculated control colour of indicator remained blue (Baeman *et al.* 2011).

Casein Hydrolysis

Many bacteria produce enzymes that hydrolyze protein. To perform this test *B. subtilis* isolate was inoculated on skim milk agar (Nutrient Agar + 2% raw skim milk) plates and incubated for 3 days. Colonies of organism which digest casein appeared surrounded by clear zones indicating a positive test. Areas in which the casein was attacked remained slightly opaque, whereas in negative test no zone formation was observed (Tariq *et al*. 2016).

Phosphate solubilization

To perform this test *B. subtilis* isolate was spot inoculated on Pikovaskaya's media. After inoculation plates were incubated for 4-5 days at 28 ± 1^0C temperature. Solubilization of phosphate was indicated by the formation of a clear inhibition zone arround the colony indicating positive test. No zone formation was observed in uninoculated control (Jadhav *et al*. 2014).

Methyl red (MR) Test

MR test was performed to check the cleavage of glucose which is commonly used in the differentiation of organisms. To perform the test Glucose Phosphate Broth media (Glucose 0.5%, K_2HPO_4 0.5%, Peptone 0.5%,

distilled water 1litre) was filled in the test tubes and sterilized. After inoculation with *B. subtilis* isolate tubes incubated for 7 days at 27±2^{0}C temperature. Five drops of the methyl red indicator (0.1g Methyl red dissolved in 300ml of 95% ethanol and made up to 500ml with distilled water) were added to 5ml of culture. Production of Red color indicated a positive test, whereas, yellow coloration was recorded as negative test (Singh *et al*. 2017).

***In vitro* efficacy of *B. subtilis* isolates**

Collected phylloplane *B. subtilis* isolates were tested for their antagonistic potential against four grain mold causing fungi of sorghum i.e. *F. moniliforme, A. alternata*, *C. lunata* and *P. sorghina*, by dual culture technique (Petkar, 2021) on PDA media along with the untreated inoculated control. Two streaks of *B. subtilis* isolate were placed 3cm apart on the media surface and 5mm disc of pathogenic fungi was placed at the centre with 3 replications for each combination. Thus inoculated Petri-plates were incubated at 27±2^{0}C for seven days. Per cent growth inhibition was calculated as per Charpe *et al.* (2017).

Statistical treatment of data

Data was subjected to analysis by Completely Randomized Design (CRD) using WASP-1.0 software of Central Coastal Agricultural Research Institute of Indian Council of Agricultural Research, Goa, India (ICAR-CCARI, 2024).

III. RESULTS

Isolation of grain mold causing fungi and pathogenicity test

Fungal cultures were isolated from grain mold infected field samples and were subjected to pathogenicity test. Amongst the collected fungal cultures *Fusarium moniliforme, Alternaria alternata, Curvularia lunata* and *Phoma sorghina* has proven the pathogenicity by molding the sorghum grains. So, they were maintained as axenic cultures on PDA for further experimentation.

Isolation of *B. subtilis* from phylloplane

Samples were collected from phylloplane during *kharif* and *rabi* seasons from sorghum, rice, cotton, soybean, pigeonpea and chilli crops etc. from various research units of Dr.PDKV, Akola, Maharashtra (Table 1). Collected samples were processed in the laboratory for isolation of *B. subtilis* on NA media by serial dilution technique. After three days of incubation, milky white colonies were observed which were later picked up and streaked on fresh NA plates for pure culture and used for further study. The data presented in Table 1 and Fig. 1 shows the crop-wise isolates of *B. subtilis* obtained from phylloplane samples of different field crops and were designated as PBs1, PBs2, PBs3, PBs4, PBs5 and PBs6, respectively.

Table 1. Isolation of phylloplane Bacillus subtilis from different crops and their morphological characterization

Crops	Locations	*B. subtilis* Isolate Number	Cell shape	Colony shape	Colony colour
Sorghum	Sorghum Research Unit, Dr. P.D.K.V., Akola	PBs1	Rod	Circular, Wet, Smooth, Concave	Dirty white
Rice	Sakoli	PBs2	Rod	Regular, Wet, Smooth, Entire margin	Dull white
Cotton	Cotton Research Unit, Dr. P.D.K.V., Akola	PBs3	Rod	Irregular, Dry, Smooth, Flat and Lobate margin	Slightly dirty white
Soybean	Pulses Research Unit, Dr. P.D.K.V., Akola	PBs4	Rod	Spreading, Wet, Smooth, Flat and Irregular with Lobate margin	Off white
Pigeonpea	Pulses Research Unit, Dr. P.D.K.V., Akola	PBs5	Rod	Circular, Wet, Smooth, Concave	Slightly white
Chilli	Chilli Research Unit, Dr. P.D.K.V., Akola	PBs6	Rod	Circular, Dry, Smooth, Flat and Irregular with Lobate margin	Transparent dirty white

Fig 1. Isolates of Bacillus subtilis on nutrient agar medium

Morphological characterization of phylloplane *B. subtilis* isolates

Bacterial cells of all the six phylloplane *B. subtilis* isolates appeared rod-shaped under 40x magnification of microscope. On NA media phylloplane *B. subtilis* developed as typical well-separated white colonies with a colour variation from creamy white to dirty white. Morphological characterization was done by classical macroscopic techniques of colour, elevation, form and shape of pure colonies. Most colonies were able to grow within 2-3 days when incubated at 25±2ºC temperature.

Biochemical characterization of phylloplane *B. subtilis* isolates

All the six phylloplane *B. subtilis* isolates were subjected to biochemical tests for their identification and further some tests were performed for comparison of the characteristics among isolates. Collected isolates were tested for shape, Gram's reaction, KOH solubility, starch hydrolysis, catalase test, gelatin liquefaction, H_2S production, casein hydrolysis, IAA production, MR test and acid & gas production.

Table 2. Biochemical properties of selected phylloplane Bacillus subtilis isolates

Sr. No	Character/ Properties	Reaction/ Isolates					
		PBs1	PBs2	PBs3	PBs4	PBs5	PBs6
1	Gram reaction	+	+	+	+	+	+
2	KOH test	-	-	-	-	-	-
3	Starch hydrolysis	+	-	+	-	+	+
4	Catalase test	+	+	+	+	+	+
5	Gelatinliquefaction	+	+	+	+	+	+
6	H_2S Production	+	+	+	+	+	+
7	Casein hydrolysis	+	+	+	+	+	+
8	Acid and Gas Production	+	+	+	+	+	+
A	Acid	+	+	+	+	+	+
B	Gas	-	-	-	-	-	-
9	IAA production	+	+	+	+	+	+
10	Phosphate solubilization	+	+	+	+	+	+
11	MR test	-	-	-	-	-	-

Fig. 2. Biochemical tests of collected Bacillus subtilis isolates

Efficacy of phylloplane *B. subtilis* isolates against sorghum grain mold fungal pathogens by dual culture technique

Data presented in Table 3, Fig. 3 and graphically represented in Fig. a, b, c and d indicated that all the isolates control the growth of *F. moniliforme*, *A. alternata, C. lunata and P. sorghina* with percent growth inhibition ranging between 65.99 – 77.09% in all six phylloplane isolates of *B. subtilis*.

Table 3. Efficacy of phylloplane Bacillus subtilis isolates against grain mold fungi of sorghum

Isolates	Host	Mean of mycelial growth of fungi in control (mm)	Mean of mycelial growth inhibition of fungi in treatment (%)
PBs1	Sorghum	29.16	62.42
PBs2	Rice	22.08	71.53
PBs3	Cotton	20.99	73.07
PBs4	Soybean	**18.75**	**75.91**
PBs5	Pigeonpea	24.17	68.84
PBs6	Chilli	25.75	66.87
Pathogen	Control	79.42	00.00

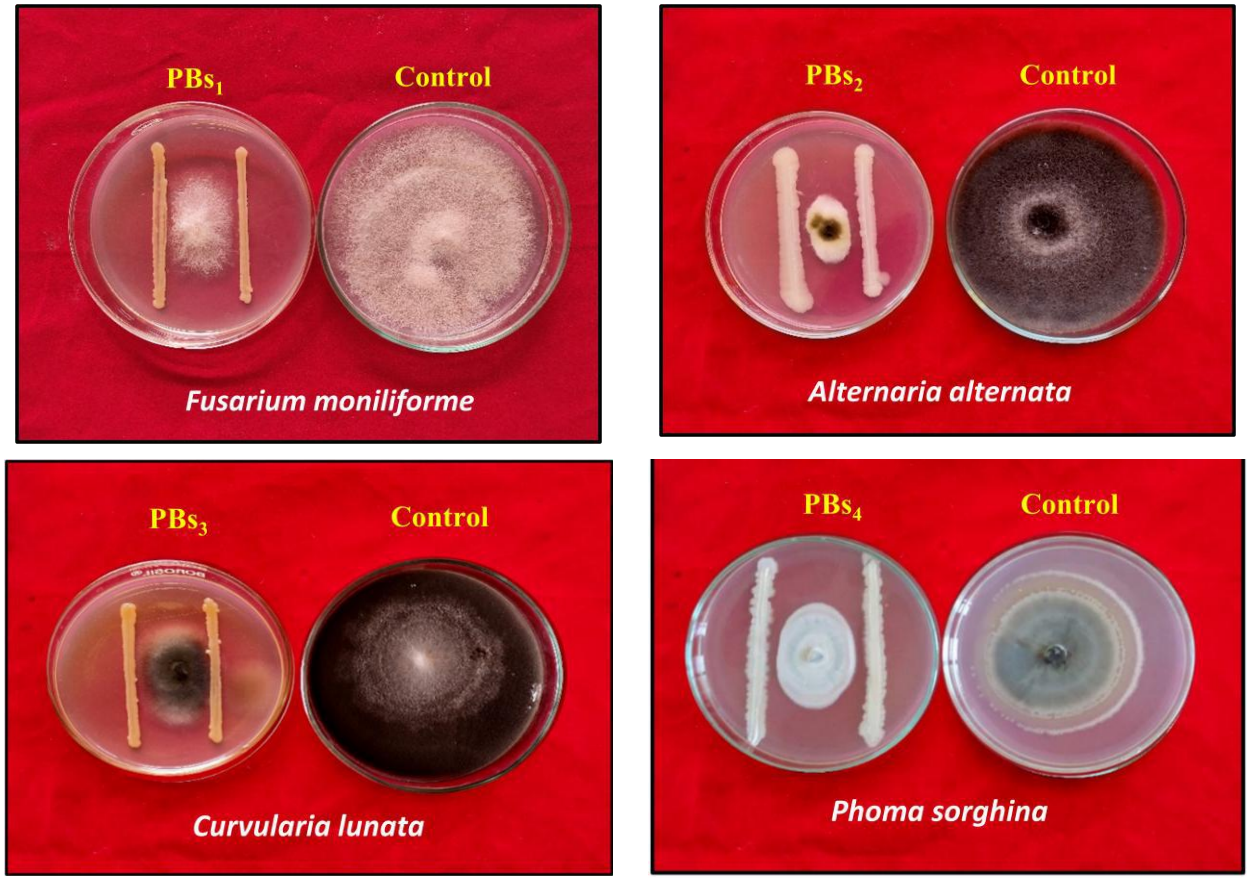


Fig 3. Efficacy of phylloplane Bacillus subtilis isolates against grain mold fungi of sorghum

IV. DISCUSSION

Isolation of grain mold causing fungi and pathogenicity test

All the four pathogenic genera *F. moniliforme, A. alternata, C. lunata* and *P. sorghina* obtained from molded grains of sorghum are earlier reported to cause Sorghum Grain Mold (SGM) Disease. Das *et al*. (2020) and Ackerman *et al*. (2021) have reported *F. moniliforme, A. alternata* and *C. lunata* as predominant pathogens responsible for SGM disease. Whereas, along with these three genera Thakur *et al*. (2006) has reported *Phoma sorghina* also as a fungal pathogen responsible for molding of sorghum grains. Thus, current findings are in agreement with the earlier reports.

Isolation of *B. subtilis* from phylloplane

The study supports the findings of earlier workers those have isolated *B. subtilis* from the phylloplane. Patro *et al.* (2002) recorded three isolates of phylloplane bacteria (Plb) (*Bacillus* spp.) from mungbean leaves. Brian (2004) isolated and reported that *B. subtilis* was the most abundant bacteria cultured from the phylloplane of soybean. Mohammadipour *et al.* (2009) studied the characterization of surfactin-producing 290 phylloplane isolates of *B. subtilis* collected from different ecological zones of Iran. Similarly, Theoduloz *et al.* (2003) reported that *B. subtilis* is a natural inhabitant of the tomato phylloplane. Pane and Zecardelli (2015) recorded 93 strains of *B. subtilis* spore-forming bacteria isolated from solanaceous phylloplane that were screened for *in vitro* antibiotic activity against *A. alternata* causal agent of tomato early blight. Sameer *et al.* (2018) had conducted studies to isolate potential phyllosphere colonizing antagonistic microbes (*B. subtilis*) for the management of *Fusarium* ear rot. Thus, the current study further confirmed the existence of *B. subtilis* in the phylloplane of different crops which have been reported by above referred workers.

Morphological characterization of phylloplane *B. subtilis* isolates

Data presented in Table-1 revealed that all *B. subtilis* phylloplane isolates were Gram-positive and rod-shaped. Similar results have been earlier reported by Perez (2000) and Toppo *et al.* (2015). These morphological characters confirm that the collected isolates were of *B. subtilis* bacteria.

Fig. (a). Efficacy of phylloplane Bacillus subtilis isolates against Fusarium moniliforme

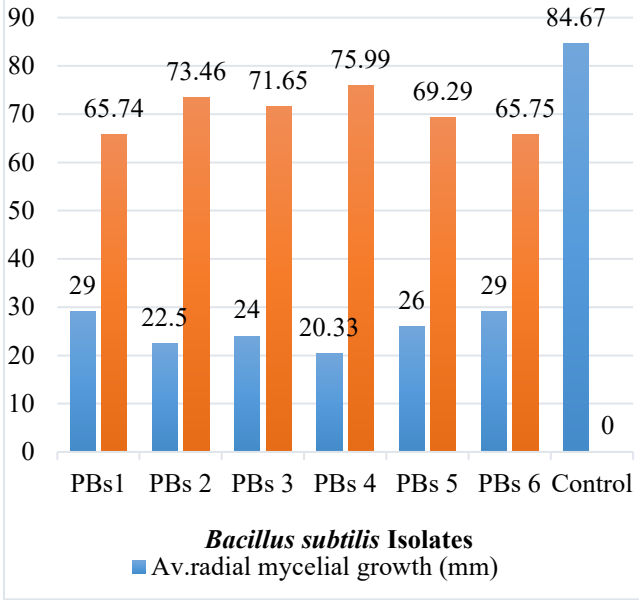


Fig. (b). Efficacy of phylloplane Bacillus subtilis isolates against Alternaria alternata.

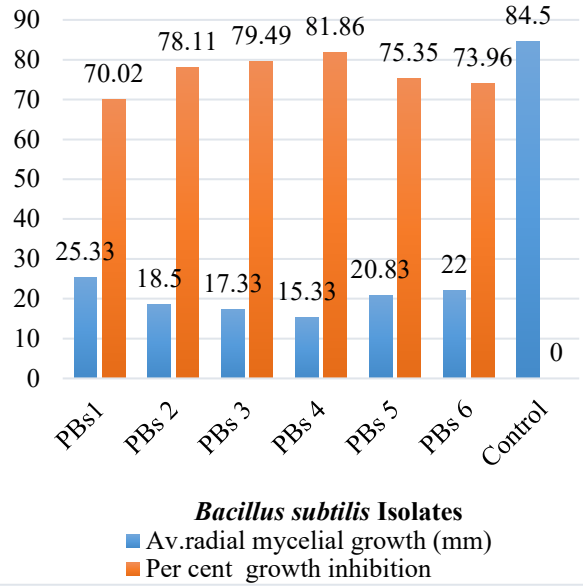


Fig. (c). Efficacy of phylloplane Bacillus subtilis isolates against Curvularia lunata

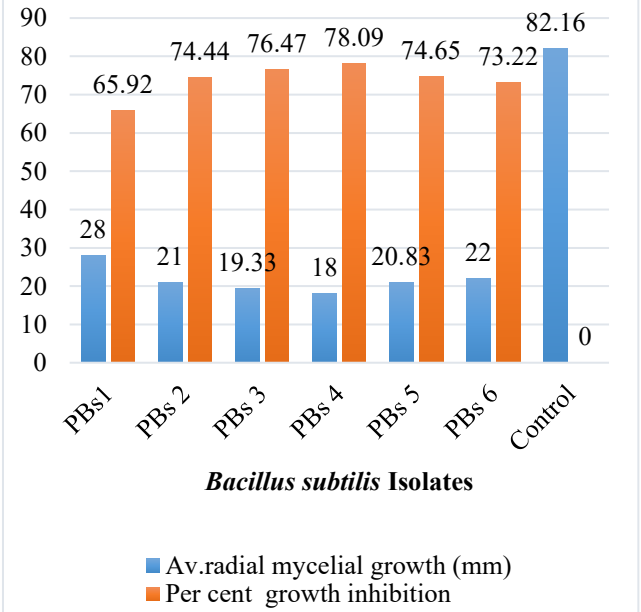
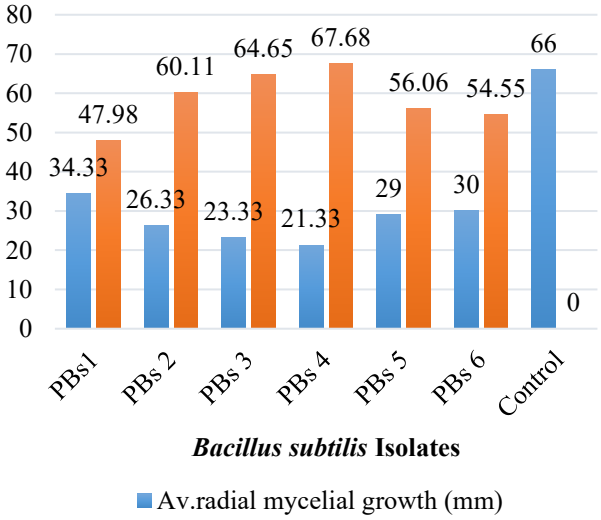


Fig. (d). Efficacy of phylloplane Bacillus subtilis isolates against Phoma sorghina



Here, minimum mycelial growth (18.75 mm) with maximum mycelial growth inhibition (75.91%) of *F. moniliforme, A. alternata, C. lunata* and *P. sorghina* was recorded in phylloplane isolate *B. subtilis* PBs4 followed by PBs3 (20.99 mm, 73.09%), PBs2 (22.08 mm, 71.53%) and PBs5 (24.17 mm, 68.84%). The other isolates of *B. subtilis* i.e.PBs6 and PBs1 were found least effective against all the fungal pathogens.

Biochemical characterization of phylloplane *B. subtilis* isolates

Data presented in Table 2 and Fig. 2 indicates that all the phylloplane *B. subtilis* isolates had positive reactions to Gram's reaction, starch hydrolysis, gelatin liquefaction, H_2S production, casein hydrolysis, catalase test, acid production and phosphate solubilization tests. Negative reaction was obtained for indole production, KOH solubility, MR test, acid & gas production and for starch hydrolysis test among the PBs2 and PBs4 isolates. This is

in agreement with the positive reactions with *B. subtilis* for starch hydrolysis, gelatin liquefaction, H_2S production, casein hydrolysis, catalase, phosphate solubilization and acid & gas production reported by Karimi *et al.* (2012), Abbo *et al*. (2014), Jadhav *et al*. (2014), Jha *et al*. (2016), Tariq *et al*. (2016). Avsar *et al*. (2017), Mandla *et al*. (2017) and Zhen xiang *et al*. (2018). Whereas, Willemse *et al.* (1980), Khan *et al.* (2011), Abbo *et al.* (2014) and Jha *et al*. (2016) has reported negative reactions regarding the KOH test, gas production, starch hydrolysis and indole production by *B. subtilis* that is also confirmed by the current results. The positive and negative results for biochemical tests of different *B. subtilis* isolates reported by the earlier workers confirmed the morphological and biochemical identification of the isolates under study.

Efficacy of phylloplane *B. subtilis* isolates against sorghum grain mold fungal pathogens by dual culture technique

The result of the present investigations is in agreement with the findings of Ghosh *et al.* (2014) who demonstrated antagonistic potential of *B. subtilis* against *F. moniliforme var. subglutinans in vitro* by dual culture plate method. They reported *Bacillus subtilis* strongly inhibited the growth of mycelium and spore germination of the pathogen and the non-volatile metabolites of *B. subtilis* had exhibited 72.00% inhibition of radial growth of fungal pathogen. Amaresan *et al.* (2012) reported *B. subtilis* isolates were effective against *Colletotrichum capsici* in chilli. Further, the suppression of mycelial growth of *C. gloeosporiodes* causing anthracnose in *Dendrobium* with crude extract of antifungal compound produced by *B. subtilis* was also reported by Prapagdee *et al.* (2012). The inhibition of *C. lindemuthianum* causing anthracnose of cowpea with different strains of *B. subtilis* (Bs-21, Bs-22 and Bs-23) was recorded by Adebanjo (2004). Laha and Venkantaraman (2001), Muralidharan *et al.* (2004) and Singh and Sinha (2004) reported inhibition of *C. lunata* causing black kernel in rice with *B. subtilis* (97.77%). Mycelial growth inhibition of *Curvularia geniculata* with antifungal compound produced by *B. subtilis* was reported by Dass and Teyegaga (1996). The strong inhibitory effect *in vitro* against *Phoma* spp. with *B. subtilis,* isolated from wheat phylloplane and suppression of *Myrothecium* spp. in watermelon with *B. pumilus* were reported by Perello *et al.* (2001) and Lokesh *et al.* (2007), respectively. These findings regarding antifungal activity of *B. subtilis* are confirmed by the results of the present investigation as well.

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