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In vitro Evaluation of Bacterial Endophytes for Biocontrol of *Pythium aphanidermatum* and Plant Growth Promotion in *Setaria italica* L. Grown in Seedling Trays

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Authors' contributions

Author MRR, is carried this study as a part of thesis work during the PhD programme at Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, India. Author MKS is Professor and major advisor to the author MRR, designed the study and protocols. Author SA helped in statistical analysis and literature searches. All authors read and approved final manuscript.

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Original Research Article

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ABSTRACT

An investigation study was carried to test the ability of endophytic bacteria isolated from small millets as a biocontrol agent against the fungal pathogen *Pythium aphanidermatum,* the causal organism of sheath blight in foxtail millet (*Setaria italica* L.) grown in seedling trays under greenhouse conditions. In total twelve bacterial endophytes were isolated out of which six isolates produced β -1, 3 glucanases, 11 isolates produced chitinases and 12 isolates produced siderophores under *in-vitro* conditions. All these bacterial endophytic isolates inhibited mycelial growth of *Pythium aphanidermatum* in plate assay and liquid culture and the highest percent inhibition of mycelium was recorded in KMS5 (44.44%) followed by KMS1 (38.89%). In seedling

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trays, BMR7 and KMS5 (81.66%) had recorded the highest percent germination followed by KMS1 (79.62%) and KMS1 and KMS5 took 10.85 and 10.55 days respectively for 50% seed germination compared to control (13.50 days) which was treated with pathogen alone. Lowest pre-emergence disease incidence was noticed in KMS5 (14.03%) followed by KMS1 (16.18%) whereas T1 (Control) recorded maximum pre-emergence disease incidence (39.82%) was observed. Least post-emergence disease incidence (11.36) and biocontrol efficacy (68.74%) was recorded with KMS5. Apart from showing antagonistic activity, KMS5 had recorded a significantly higher seedling vigor index (2712.97), root length (18.12 cm), shoot length (15.10 cm), root dry weight (0.158 g) and shoot dry weight (0.76 g) compared to other bacterial endophytic isolates.

Keywords: Bacterial endophytes; foxtail millet; Pythium aphanidermatum; antagonistic activity.

1. INTRODUCTION

Endophytes that reside inside the plant tissues not only take care of its host plant health but also fight against the plant pathogens which can be extended its usage as bioinoculants for field crops. Antagonistic activity exhibited by bacterial endophytes makes them suitable for the use of biocontrol agents and these endophytes colonize actively in the host plant tissues and establish a beneficial lifelong active association with the plant without harming them [1,2,3,4,5,6]. The endophytic microbial community finds its entry into the host tissues from the rhizosphere zone in the soil [7]. Biocontrol mechanisms exhibited by bacterial endophytes includes antibiosis, siderophore productions, lytic enzymes inducing host defense mechanisms (induced systemic resistance, ISR), parasitism, competition and signal interference by quorum sensing and occupying ecological niches in the host tissues [8,9,10,11,12].

Millets belong to the Poaceae (Graminae) family of the monocotyledon group and India is considered as the major hub for these minor crops [13,14]. Small millets represent many species but, six species are predominantly known for their cultivation in India and other parts of the world. The prominent six species include finger millet (*Eleusine coracana* L.), little millet (*Panicum sumatrance*), kodo millet (*Paspalum scrobiculatum* L.), foxtail millet (*Setaria italic* L.), barnyard millet (*Echinochloa frumentacea* L.) and proso millet (*Panicum miliaceum* L.). These millet crops have been cultivated in dryland areas from ancient days in Indian and other parts of the world. Millets are more nutritious than cereals and are rich in fiber, iron, magnesium, phosphorous and potassium and have a balanced amino acid profile and are called nutria-cereals [15,16,17]. Water requirement for millets is very less and can grow effectively under scarce rainfall zones and drylands [18]. In India, they are grown in many states in a variety of agro-ecological zones v*iz*., plains, hills, and coasts as well as in diverse soils under different rainfall conditions [19].

Among small millets, foxtail millet (*Setaria italica*. L) is the second most widely grown crop in India and rich in nutrients. Foxtail millet (*Setaria italica*.L) called by different vernacular names such as German, Italian, Siberian millet [16]. At present, in India, its cultivation is confined to semi-arid regions in the states of Andhra Pradesh, Karnataka, Chhattisgarh and Tamil Nadu. Damping-off causing *Pythium aphanidermatum* in millets was reported from Annamalai in Tamil Nadu, India [20]. This fungal pathogen grows vigorously and produces symptoms in bad drained nurseries/fields, especially during rainy months. Disease symptoms starts in hypocotyl region at the ground level include the appearance of yellowish-brown discoloration and spread to roots as well as stems and finally, the seedlings collapse.

The present investigation study was carried to evaluate the efficiency of endophytic bacteria on biocontrol of *Pythium aphanidermatum* in foxtail millet grown in seedling trays under greenhouse conditions.

2. MATERIALS AND METHODS

2.1 Sampling and Isolation of Bacterial Endophytes

Samples of complete millet plants (Barnyard, foxtail, finger, kodo, little and proso millet) were collected during Kharif and Rabi seasons of 2016-17 from the millet research plots at ZARS, University of Agricultural Sciences, GKVK, Bengaluru, India (12.58° North latitude and 77.58° East longitude). From each plant, leaf segments, shoot segments, and root segments

(ten samples each) were immersed in 70% ethyl alcohol for two minutes to remove adherent surface microflora. After washing with distilled water, the plant samples were immersed and shaken in 1.2% (v/v) sodium hypochlorite solution for 15-20 minutes followed by washing with sterile distilled water for 5-6 times to remove traces of the chemical. Surface sterilized plant samples were made into 1-2 cm pieces by cutting on either side of root, shoot and leaf samples to maintain uniformity in sizes of plant parts. These 1-2 cm plant parts were placed vertically on plates containing fresh tryptic soy agar (TSA) medium (Himedia laboratories, India) and incubated at 28±2°C for 2 days. Bacterial endophytes present inside plant tissues come out along with oozing sap and form colonies on the TSA plates and the bacterial isolates were purified on fresh nutrient agar medium. The purified bacterial endophytic isolates were stored at -80°C in 15% (v/v) glycerol stock solutions for further studies [2].

2.2 Siderophore Production

The siderophore producing endophytic bacterial isolates were assessed on King's B medium containing chrome azural dye [21].

2.2.1 Preparation of Chrome Azural S (CAS) solution

Chrome Azural S solution was prepared by dissolving 60.5 mg of chrome azural S in 40 ml of double distilled water and mixed with 10ml of ferric chloride (1mM) solution in HCl (10 mM). The solution added to a 40 ml aqueous solution containing 72.90 mg of cetyl trimethyl ammonium bromide (CTAB) and the final solution was sterilized at 121°C at 15psi for 30 min.

2.2.2 Media preparation

King's B agar medium containing piperazine N, N-bis 2-ethane sulphuric acid (PIPES) (30.2 g/L) was prepared and adjusted to a pH of 6.80 and autoclaved. After cooling, the CAS solution dye (100 ml) was added to the medium. The Chrome azural S medium (CAS agar) was poured to the petri plates and refrigerated (4ºC) overnight. Purified endophytic bacterial isolates were streaked on the agar plates and were incubated in a BOD incubator at 28±2ºC for 24 h. After incubation, the plates with bacterial colonies were observed for a yellow halo around the bacterial colony, which indicates positive for siderophore production.

2.3 Biocontrol Mechanisms of Bacterial Endophytic Isolates against Fungal Pathogens

2.3.1 β-1, 3-glucanase assay

β-1, 3-glucanase production by the bacterias was estimated by the laminarin dinitro salicylic acid method [22]. Overnight grown bacterial cultures were transferred to a test tube containing 2 ml of 0.05M sodium acetate buffer (pH 5.0) and centrifuged at 16000 g for 15 min at 4ºC. 62.5 µl of 4% laminarin and 62.5µl of culture extract were added to a test tube and kept at 40°C for 10 min. Then enzyme action was then stopped by adding 375 µl of dinitro salicylic acid followed by heating for 5 min on boiling water bath, vortexed and absorbance was recorded at 500 nm using purified β-glucanase as a standard and the enzymatic activity was expressed as nmol glucose released min^{-1} ml^{-1}.

2.3.2 Chitinase assay

The bacterial endophytic cultures were homogenized in sodium citrate buffer (2 ml of 0.1 M at pH 5.0) and centrifuged at 16000 rpm for 15 min at 4ºC and the supernatant was used for enzyme assay. Colloidal chitin is used as a substrate for the colorimetric assay of chitinase [23]. The reaction mixture contains 0.1 ml of colloidal chitin (10 mg), 10 µl of 0.1M sodium acetate buffer (pH 4.0) and 0.4 ml enzyme extract. The reaction mixture was kept for incubation for 2 h at 37ºC. After the incubation period, the enzyme reaction was stopped by centrifugation at 1000 rpm for 3 min and an aliquot of 0.3 ml was pipetted out into a glass tube having 30 µl potassium phosphate buffer (pH 7.0, 1M) and 20µl 3% (w/v) snail gut enzyme was incubated at 37ºC for 1 h. After incubation pH of the reaction mixture was maintained at 8.9 by adding 70 µl of 0.1M sodium borate buffer and was placed on a boiling water bath for 30 minutes and immediately cooled by placing on ice cubes. Then 2 ml of p-dimethyl amino benzaldehyde (DMAB) was added to the reaction mixture and incubated at 37ºC for 20 minutes and absorbance was measured at 585 nm. A standard graph was plotted by using N-Acetyl glucosamine (GlcNAc) as standard and the activity of enzyme was expressed as nmol GlcNAc equivalents $min^{-1}ml^{-1}$.

2.4 Percent Inhibition of Fungal Pathogen

Antifungal activity was screened by using a dual culture plate method on Potato Dextrose Agar

(PDA) media. The test fungus, *Pythium aphanidermatum* was obtained from Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru, India. Further, the inhibition zone was measured and the percent inhibition of the pathogen (*Pythium aphanidermatum*) was calculated by using the formula mentioned below

$$I = \frac{(C-T)}{C} \times 100$$

Where, I = Percent inhibition, C = Growth of fungal plant pathogens in control (mm),
T = Growth of fungal plant pathogens in dual culture plate (mm).

2.5 Evaluation of Bacterial Endophytic Isolates on Growth Inhibition of *Pythium aphanidermatum* in Liquid Culture

The bacterial endophytes showing the highest pathogen inhibition in plate assay were tested in liquid media (Potato dextrose broth). Mycelial discs (5 mm) of *Pythium aphanidermatum* was inoculated to liquid broth along with one ml of 24 hour old bacterial endophytes with three replications each [24]. Control flask without any bacterial endophyte addition was maintained and all flasks were incubated in a BOD incubator at 28±2ºC under static conditions for 10 days and the contents in the flasks were filtered through a pre-weighed Whatman filter paper and fresh weight of contents were recorded. The filter papers along with contents were dried in a hot air oven at 105ºC for 48 hr and again reweighed along with the mycelium to obtain constant dry weight values. The fungal mycelial weight was calculated by subtracting the weight of the pre-weighed filter paper from the weight of the filter paper + mycelial mat. The reduction in mycelial weight in co-inoculated flasks was determined by comparing the weights obtained from the control flasks containing only the fungal pathogen.

2.6 Seedling Tray Experiment

Seedling tray experiment was conducted in the greenhouse facilities located in Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru. The substrate for the experiment included 10 kilograms of coir pith enriched with 2.5 kilograms of red earth, vermicompost and pongamia cake each, which were autoclaved before using for the experiment. Isolated bacterial endophytic cultures were grown in nutrient broth and added @ 10 ml/kg of substrate.

2.6.1 Preparation of pathogen inoculums

Pathogen inoculums were prepared by transferring agar discs (5 mm) of *Pythium aphanidermatum* to the sterile polybags containing a mixture of sand and crushed sorghum (94:6) and incubated at 27±1°C for 15 days.

2.6.2 Preparation of seedling trays and sowing

The mass multiplied pathogen inoculum of *Pythium aphanidermatum* was added to substrate mixture @ 100 g/kg to each polybag and bacterial endophytes were added @ 100 ml/kg of the seedling mixture and mixed properly one week prior to sowing. The mixed substrate was added @ 100 g/tray at the time of sowing.

2.6.3 Observations recorded

During the experiment germination percentage, shoot and root length, seedling vigor index [25], percent pre and post-emergence disease incidence and biological control efficacy [26] were recorded.

2.7 Statistical Analysis

All treatments were replicated thrice and the experimental data generated in lab studies and seedling tray studies were subjected to one-way ANOVA [27]. Means were separated by Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1 Isolation, Purification and Naming of Bacterial Endophytic Isolates

Twelve endophytic bacteria were isolated from the millet samples from the three different plant parts. And these isolates were purified on fresh Nutrient agar plates by quadrant plate streaking method. These isolated bacteria were named as follows

1st letter indicates the small millet name
2nd letter "M" indicates the name "Millet" and
3rd letter indicates the plant part (S for shoot, R for root and L for leaf) and the number indicates the isolate number.

E.g. FMR7 indicate Finger Millet Root Isolate 7.

3.2 Biocontrol Activities of Bacterial Endophytic Isolates

3.2.1 Siderophore production

In the present study, twelve bacteria were isolated and all isolates shows positive for siderophore production under *in-vitro* conditions (Table 1). Several studies on siderophore producing bacteria significantly influenced the uptake of various nutrients including iron (Fe^{+2}), zinc (Zn^{+2}) and copper (Cu^{+2}) by plants [28,29,26]. Siderophores namely pyoverdine from *Pseudomonas fluorescens*; catechols from Enterobacteriaceae [30] directly stimulates synthesis of other antimicrobial compounds required for induced systemic resistance (ISR), antibiosis and also increase the availability of minerals to the rhizospheric bacteria thereby suppressing the growth and survival of pathogenic fungi *viz., Fusarium oxysporum and Rhizoctonia solani* [31,32,33].

3.2.2 β-1,3-glucanase production

The role of β-1, 3-glucanase in cell wall degradation and preventing fungal plant pathogen growth is well understood and microbes producing these β-1,3-glucanase can be used as effective biocontrol agents. Out of twelve bacterial isolates, six were shown positive for β-1, 3-glucanase production. The isolate BML1 (12.45 µmol/min/ml) recorded significantly higher β-1, 3-glucanase production followed by LML4 (10.45 µmol/min/ml). The lowest concentration of β-1, 3-glucanase was observed in BMR7 (6.45 µmol/min/ml). β-1, 3-glucanase produced by *Paenibacillus* sp. and *Streptomyces* sp. degraded the fungal cell walls of *F. oxysporum* f. sp. *cucumerinum*. Some studies showed that *Burkholderia cepacia* synthesized β-1, 3-glucanase and this bacterium destroyed the cell walls of *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium ultimum* [34,35]. Extracellular enzymes like β-1,3-glucanases and chitinase production from fluorescent Pseudomonad isolates GRC_3 and GRC_4 against pathogenic fungi *Phytophthora capsici* and *Rhizoctonia solani* also reported that these extracellular enzymes and antifungal metabolites were the major inhibitors of the growth of *P. capsici* and *R. solani* [36].

3.2.3 Chitinase production

The most commonly reported mechanism for biocontrol activity by rhizospheric bacteria and endophytic bacteria is antagonism through predation, competition, and production of enzymes or chemicals which are the antagonist in nature. Unlike rhizospheric microbes, the endophytes have an alternative mechanism of biocontrol known as induced systemic resistance (ISR) where metabolites of the bacteria affect the host plant to get resistance against the pathogens. Chitinase enzyme produced by the endophytic microorganisms degrades the fungal pathogen haustorium, through which the pathogen absorbs nutrients from the host plants and in turn plays a major role in biological control of fungal diseases [37].

Among twelve bacterial endophytes, eleven isolates were shown positive for chitinase production (Table 1). KMS5 (9.56 µmol/min/ml) recorded highest chitinase production followed by KMS1 (9.51 µmol/min/ml) and BML1 (9.34 µmol/min/ml). The lowest chitinase production was recorded in BMR7 (8.78 µmol/min/ml). Similar results were also reported with the crude culture filtrates of *Actinoplanes missouriensis*, an endophyte isolated from lupin crop produce high levels of chitinase and digest the hyphae of *Plectosporium tabacinum* [37]. Frankowski et al. [38] reported that the chitinase enzyme produced by *Serratia plymuthica* from oil seed rape was effective on *Verticillium dahlia, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Some researchers reported that *Serratia plymuthica* inhibited spore germination and germ-tube elongation in *Fusarium solani* and also reported that extracellular enzymes like chitinase and laminarinase produced by *Pseudomonas stutzeri* digested and lysed mycelium of *F. solani* [39].

3.3 Antagonistic Activity of Bacterial Endophytic Isolates on the Growth of *Pythium aphanidermatum*

All the twelve isolates were carried forward for testing the antagonistic activity against fungal pathogen *Pythium aphanidermatum* based on siderophore production, β-1,3-glucanase and chitinase activity (Table 1). Twelve isolates *viz.,* BMR7, BML1, FMR7, FMR12, FTMS4, FTMS5, KMS1, KMS5, LMR4, LML4, PMR6 and PML3 shown antagonism against *Pythium aphanidermatum* both in plate assay and liquid culture medium.

The isolate KMS5 had shown significantly higher percent inhibition (44.44%) in dual culture plate assay followed by KMS1 (38.89%). The lowest percent inhibition was recorded by FTMS5

(25.55%). Bacteria isolated from the surface of potato tubers and its rhizospheric soils were shown effective biocontrol of *Rhizoctonia solani* having percent inhibition ranged from 59.4 to 95.0% [40]. Similarly, a diazotrophic bacterial endophyte, *Lysinibacillus sphaericus* from rice showed 100% growth inhibition of *Rhizoctonia solani* compared to fungicide treatments and this bacterium secreted volatile organic compounds, siderophores, biosurfactants, hydrogen cyanide (HCN) and ammonia that helped in fungal pathogen suppression under greenhouse conditions [41]. Foliar and soil application of *L. sphaericus* in rice reduced the disease incidence caused by *Rhizoctonia solani* in field conditions [41]. Bacterial endophyte isolated from Korean rice shows an antagonistic effect against *Rhizoctonia solani* in a plate assay [42].

3.4 Effect of Bacterial Endophytic Isolates on Inhibition of Mycelial Growth of *Pythium aphanidermatum* in Liquid Culture

The highest percent reduction of mycelium was shown by KMS5 and PMR6 (86.58%) and was at par with FTMS4 and LML4 (85.36%) in liquid culture (Table 2). KMS1 and LMR4 recorded the highest percent reduction in dry weight of mycelium (84.14%) and the isolates FTMS4, LML4, KMS1 and LMR4 shown no significant difference in the reduction of mycelial growth. The least percent reduction in dry weight of mycelium was recorded with BMR7 (71.95%).

3.5 Biocontrol Efficacy of Bacterial Endophytic Isolates on *Pythium aphanidermatum* in *Setaria italica* Grown in Seedling Trays under Greenhouse Condition

Data related to the effectiveness of biocontrol by isolated bacterial endophytes on *Pythium aphanidermatum* in foxtail millet grown in seedling trays under greenhouse conditions were given in Table 3 and Fig. 3. The germination was maximum (81.66%) in Treatment T9 (*P. aphanidermatum* + KMS5) and T_2 (*P. aphanidermatum* + BMR7) which were statistically similar, followed by T_8 (*P. aphanidermatum* + KMS1) which recorded 79.62%. Control T_1 (*P. aphanidermatum*) shown the lowest germination (57.16%). Considerable differences were reported between the treatments regarding days taken for 50 percent germination of seedlings. The treatments T_9 (*P. aphanidermatum* + KMS5) (10.55) recorded lowest number of days taken for 50 percent germination followed by T_8 (*P. aphanidermatum* + KMS1) which recorded 10.85 days and were statistically alike with each other. T_{10} (*P. aphanidermatum* + LMR4) and T_{11} (*P. aphanidermatum* + LML4) took 11.25 and 11.35 days respectively for 50 percent germination and both were statistically alike with each other. Uninoculated control without bacterial endophyte (T_1) recorded maximum days (13.50) taken for 50 percent germination.

Lowest pre-emergence disease incidence (14.03%) was observed with T_9 and T_2 treatments were statistically alike and were followed by T_8 (16.18%), T_5 (18.76%) and T_4 (19.88%). Control (T_1) recorded 39.82% of pre-emergence disease incidence. Treatment T_9 (*P. aphanidermatum* + KMS5) recorded the lowest post-emergence disease incidence (11.36%) which was significantly lesser than T_8 (*P. aphanidermatum* + KMS1) which recorded 13.63%. Maximum post-emergence disease incidence (36.36%) was noticed in control (T_1).

Biocontrol efficiency was recorded for all treatments supplemented with bacterial endophytes and treatment T_9 was recorded with maximum biocontrol efficiency (68.74%) which was considerably higher than T_8 (62.50%). The highest biocontrol efficiency of T_9 was attributed to the use of KMS5 isolate which suppressed the disease. Biocontrol efficiency was lowest in control which may be attributed due to lack of any bacterial endophyte in the treatment to control the disease. Application of these bacterial endophytic strains for plant growth-promoting activity (PGPA) to the field crops may enhance crop productivity either by nitrogen fixation and uptake of phosphorus to the plants or by protecting plants from disease-causing fungi. The results clearly indicate that the bacterial endophytes producing extracellular cell wall degrading enzymes like glucanases, pectinases and chitinases could be used as biocontrol agents and also replaces fungicide to some extent to control *Pythium* and other soil borne diseases in a sustainable model of agriculture. These endophytic isolates also have the potential ability to inhibit pathogenic fungi and plant growth-promoting activities, which are useful attributes for sustainable agriculture in nutrient fewer soils of arid and semi-arid regions. Similarly, *Actinoplanes companulatus, Micromonospora chalcea* and *Streptomyces spiralis* isolated from cucumber roots and

reported that these organisms promoted plant growth and protected seedlings and mature cucumber plants from the diseases caused by *Pythium aphanidermatum*, under greenhouse conditions and results indicated that the three selected actinomycete isolates colonized cucumber roots endophytically for 8 weeks promoted plant growth and suppressed pathogenic activities of *P. aphanidermatum* on seedlings and mature cucumber plants [43]. Unlike rhizospheric microbes, the bacterial endophytes have an alternative biocontrol mechanism like induced systemic resistance (ISR), antibiosis, production of extracellular enzymes (glucanases and chitinases) to protect their host plants from fungal invasions and helps the host plant for acquiring disease resistance [37].

3.6 Efficacy of Bacterial Endophytic Isolates on *Pythium aphanidermatum* in Enhancing Seedling Vigor of *Setaria italica* Grown in Seedling Trays under Greenhouse Condition

The bacterial endophytes application against the pathogen (*Pythium aphanidermatum*) infested soil, individually reduced damping-off disease in Foxtail millet. The data corresponding to the efficacy of the isolated bacterial endophytes on *Pythium aphanidermatum* in enhancing seedling vigor of Foxtail millet was given in Table 4 and Fig. 4.

Treatment T_9 (*P. aphanidermatum* + KMS5) recorded the highest length of root followed by T_8 (*P. aphanidermatum*+ KMS1) recorded as 18.12 cm and 17.12 cm respectively. Control (T_1) (12.21 cm) recorded the lowest length of root which was treated with pathogen alone.

Treatment T_9 (15.10 cm) registered maximum length of shoot followed by T_8 (14.70 cm), were statistically alike with each other. The lowest shoot length was reported in control T_1 (9.10 cm). The highest root dry weight was observed in treatment T_9 (0.158 g) followed by T_8 (0.149 g). Control (T_1) recorded the least root dry weight of 0.124 g.

The dry weight of shoot was noticed highest in treatment T_9 (0.76 g) followed by treatment T_8 (0.75 g) and there was no considerable difference between treatments T_9 andT_8. Control (T_1) (0.51 g) recorded the lowest shoot dry weight.

The highest seedling vigor index of Foxtail millet was recorded with T_9 (2712.97) followed by T_8 (2533.66) which were considerably different from other treatments. There was no considerable difference among other treatments treated with bacterial endophytes. Plants treated with the pathogen alone (T_1) recorded the lowest vigor index (1218.22). Higher growth parameters were observed in treatments T_9 and T_8 attributed to biocontrol mechanisms and plant growth-enhancing activity by KMS5 and KMS1 isolates. Some studies reported that the application of *Pseudomonas fluorescens* in chilli crop reduced the damping-off disease incidence caused by *Pythium aphanidermatum* with the production of siderophores, salicylic acid and hydrogen cyanide [44,45] and *Lysobacter enzymogenes*

Table 1. Biocontrol activities of bacterial endophytes isolated from small millets

Sl. no	Bacterial Endophytic Isolates	Siderophore production	B 1,3, Glucanase Production (µ mol/min/ml)	Chitinase Production (µ mol/min/ml)
1	BMR7	+	6.45^{d}	8.78^{d}
2	BML1	+	12.45^{a}	9.34^{ab}
3	FMR7	+	6.75^{d}	8.34^{e}
4	FMR12	+	-	9.25^{bc}
5	FTMS4	+	-	7.45^{f}
6	FTMS5	+	-	8.98^{cd}
7	KMS1	+	-	9.51
8	KMS5	+	8.67^{c}	9.56^{a}
9	LMR4	+	-	8.33
10	LML4	+	10.45^{b}	-
11	PMR6	+	-	8.33
12	PML3	+	8.43^{c}	7.48^{f}

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Table 2. Antagonistic activity of endophytic bacterial isolates on growth of *Pythium aphanidermatum* in solid and liquid medium

Sl. no	Bacterial Endophytic Isolates	Percentage of inhibition (%)	Percentage of reduction in dry weight of mycelium (%)
1	BMR7	27.77^{g}	71.95^{e}
2	BML1	30.00^{f}	74.39^{e}
3	FMR7	32.22^{e}	78.05^{d}
4	FMR12	27.77^{g}	80.49^{cd}
5	FTMS4	26.66^{h}	85.36^{ab}
6	FTMS5	25.55^{i}	82.93^{bc}
7	KMS1	38.89^{b}	84.14^{ab}
8	KMS5	44.44^{a}	86.58^{a}
9	LMR4	33.33^{d}	84.14^{ab}
10	LML4	35.55^{c}	85.36^{ab}
11	PMR6	33.33^{d}	86.58^{a}
12	PML3	32.22^{e}	79.26^{d}

Note: Means with different superscript, in a column differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Table 3. Biocontrol efficacy of bacterial endophytes on *Pythium aphanidermatum* in Foxtail millet grown in seedling trays under greenhouse condition

Treatments	Percentage Germination (%)	Days taken for 50 percent germination	Pre-emergence disease Incidence (%)	Post – emergence disease incidence (%)	Biocontrol efficiency (%)
T_1 (*P. aphanidermatum*)	57.16^{h}	13.50^{a}	39.82^{a}	36.36^{a}	$0.00\ (0.100)^{j}$
T_2 (*P. aphanidermatum* + BMR7)	81.66^{a}	12.50^{b}	14.03^{j}	22.72^{e}	$37.49\ (6.13)^{f}$
T_3 (*P. aphanidermatum* + BML1)	75.21^{cd}	12.25^{bc}	20.82^{f}	27.27^{c}	$24.99\ (4.999)^{h}$
T_4 (*P. aphanidermatum* + FMR7)	76.11^{cd}	11.85^{def}	19.88^{g}	21.81^{f}	$39.99\ (6.324)^{e}$
T_5 (*P. aphanidermatum* + FMR12)	77.17^{bc}	12.15^{bcd}	18.76^{h}	15.45^{h}	$57.50\ (7.583)^{c}$
T_6 (*P. aphanidermatum* + FTMS4)	73.75^{de}	11.55^{fg}	22.37^{e}	23.63^{d}	$34.99\ (5.915)^{g}$
T_7 (*P. aphanidermatum* + FTMS5)	69.42^{f}	11.65^{efg}	26.93^{c}	27.27^{c}	$24.99\ (4.999)^{h}$
T_8 (*P. aphanidermatum* + KMS1)	79.62^{ab}	10.85^{h}	16.18^{i}	13.63^{i}	$62.50\ (7.905)^{b}$
T_9 (*P. aphanidermatum* + KMS5)	81.66^{a}	10.55^{h}	14.03^{j}	11.36^{j}	$68.74\ (8.291)^{a}$
T_{10} (*P. aphanidermatum* + LMR4)	75.83^{cd}	11.25^{g}	20.17^{fg}	27.27^{c}	$24.99(4.999)^{h}$
T_{11} (*P. aphanidermatum* + LML4)	69.42^{f}	11.35^{g}	26.93^{c}	31.81^{b}	$12.49\ (3.534)^{i}$
T_{12} (*P. aphanidermatum* + PMR6)	71.46^{ef}	11.95^{cde}	24.78^{d}	27.27^{c}	$24.99\ (4.999)^{h}$
T_{13} (*P. aphanidermatum* + PML3)	66.15^{g}	12.35^{b}	30.37^{b}	18.17^{g}	$49.99\ (7.070)^{d}$

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT).

Figures in parenthesis indicate the $\sqrt{x}$ + 0.5 transformed values

Table 4. Effect of bacterial endophytes on *Pythium aphanidermatum* in enhancing seedling vigour of Foxtail millet grown in seedling trays under greenhouse condition

Treatments	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight(g)	SVI*
T_1 (*P.aphanidermatum*)	12.21^{h}	9.10^{j}	0.124^{i}	0.51^{e}	1218.22^{f}
T_2 (*P. aphanidermatum* + BMR7)	16.50^{cd}	10.00^{i}	0.141^{def}	0.73^{b}	2164.16^{d}
T_3 (*P. aphanidermatum* + BML1)	17.20^{b}	12.50^{g}	0.138^{fg}	0.72^{b}	2233.88^{d}
T_4 (*P. aphanidermatum* + FMR7)	15.70^{ef}	11.00^{h}	0.145^{bcd}	0.67^{c}	2032.22^{e}
T_5 (*P. aphanidermatum* + FMR12)	15.56^{f}	13.00^{f}	0.135^{gh}	0.68^{c}	2204.12^{d}
T_6 (*P. aphanidermatum* + FTMS4)	14.53^{g}	13.50^{e}	0.126^{i}	0.58^{d}	2067.21^{e}
T_7 (*P. aphanidermatum* + FTMS5)	16.23^{cde}	13.40^{ef}	0.143^{cde}	0.68^{c}	2056.81^{e}
T_8 (*P. aphanidermatum* + KMS1)	17.12^{b}	14.70^{ab}	0.149^{b}	0.75^{a}	2533.66^{b}
T_9 (*P. aphanidermatum* + KMS5)	18.12^{a}	15.10^{a}	0.158^{a}	0.76^{a}	2712.97^{a}
T_{10} (*P. aphanidermatum* + LMR4)	16.75^{bc}	14.10^{cd}	0.144^{cde}	0.73^{b}	2339.46^{c}
T_{11} (*P. aphanidermatum* + LML4)	15.80^{ef}	13.80^{de}	0.139^{efg}	0.69^{c}	2054.73^{e}
T_{12} (*P. aphanidermatum* + PMR6)	16.20^{de}	14.20^{cd}	0.147^{bc}	0.67^{c}	2172.33^{d}
T_{13} (*P. aphanidermatum* + PML3)	16.42^{cd}	14.49^{bc}	0.132^{h}	0.71^{b}	2045.36^{e}

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT).
** - SVI – Seedling Vigour Index*

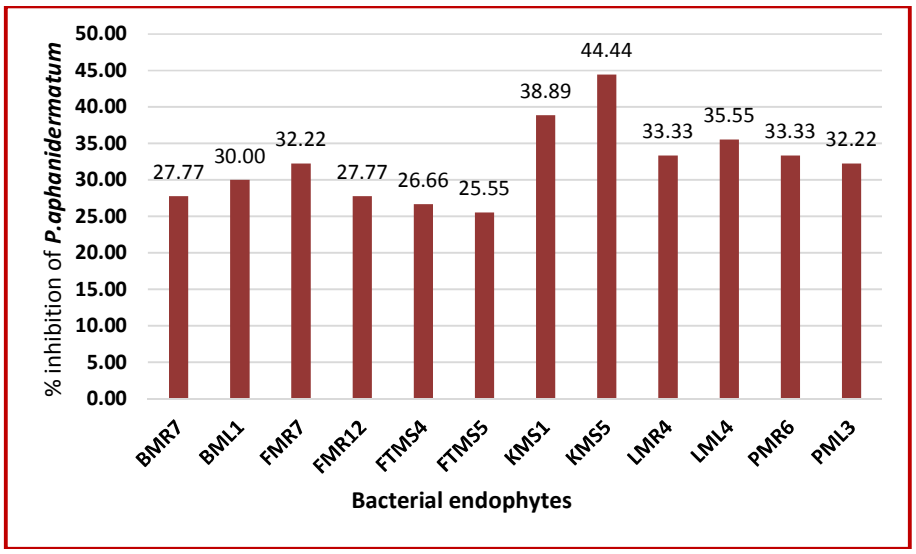

Fig. 1. Antagonistic activity of endophytic bacterial isolates on growth of *Pythium aphanidermatum* in solid medium

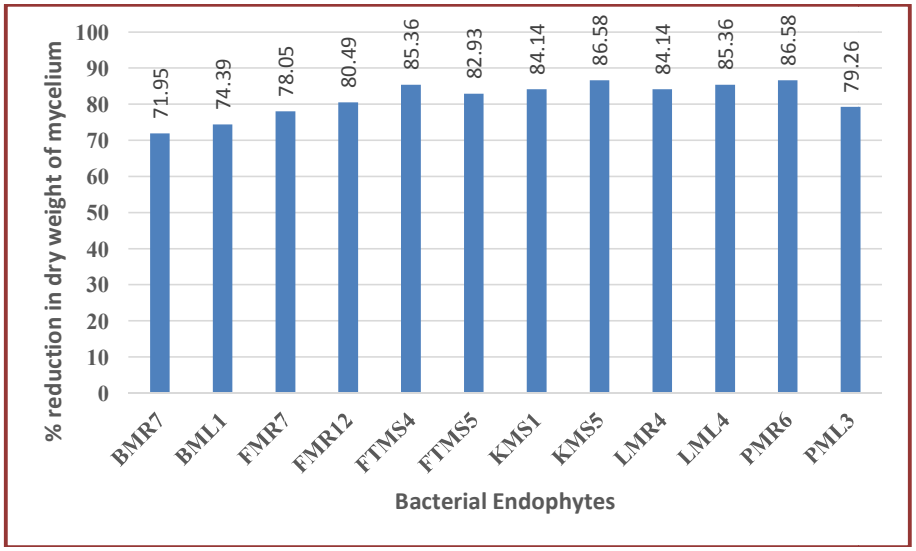

Fig. 2. Percent reduction in dry weight of mycelium of *Pythium aphanidermatum* in liquid medium

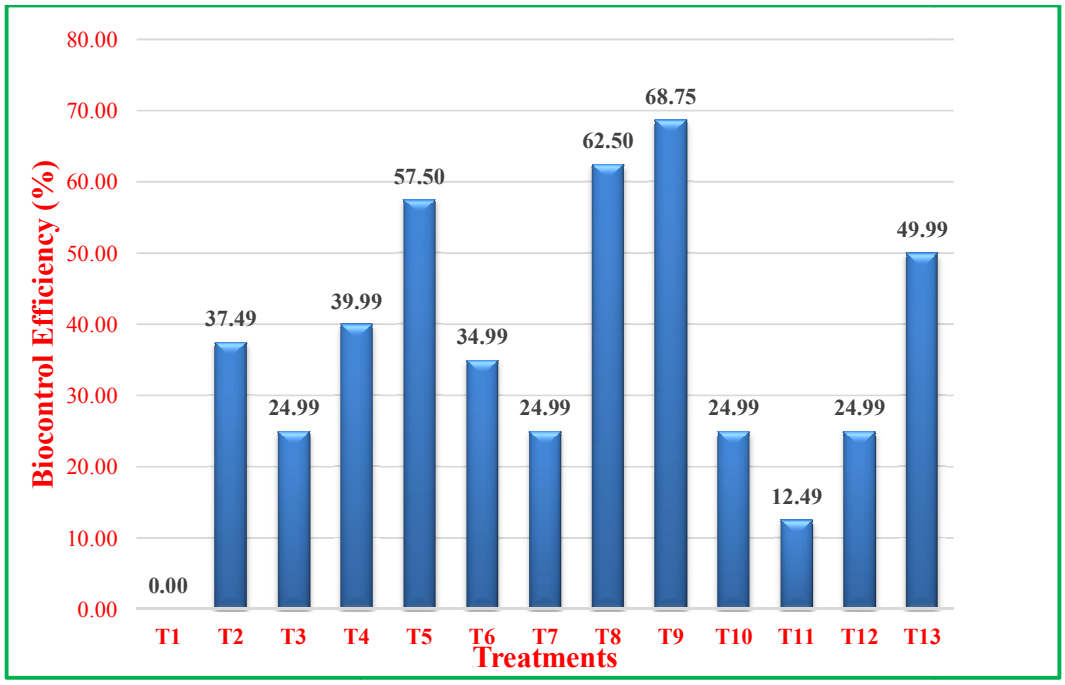

Fig. 3. Biocontrol Efficacy of endophytic bacterial isolates against *Pythium aphanidermatum* in *Setaria italica* grown in seedling trays under greenhouse conditions

isolated from rhizosphere soil samples were used for the biocontrol of *P. aphanidermatum* [46] and also application of *L. enzymogenes* along with chitosan reduced disease incidence by 50 to 100% [47]. Application of biocontrol strain *Pseudomonas fluorescens* along with arbuscular mycorrhizal (AM) fungus (*Glomus mosseae*) in tomato plants, significantly increased seedling vigor [48].

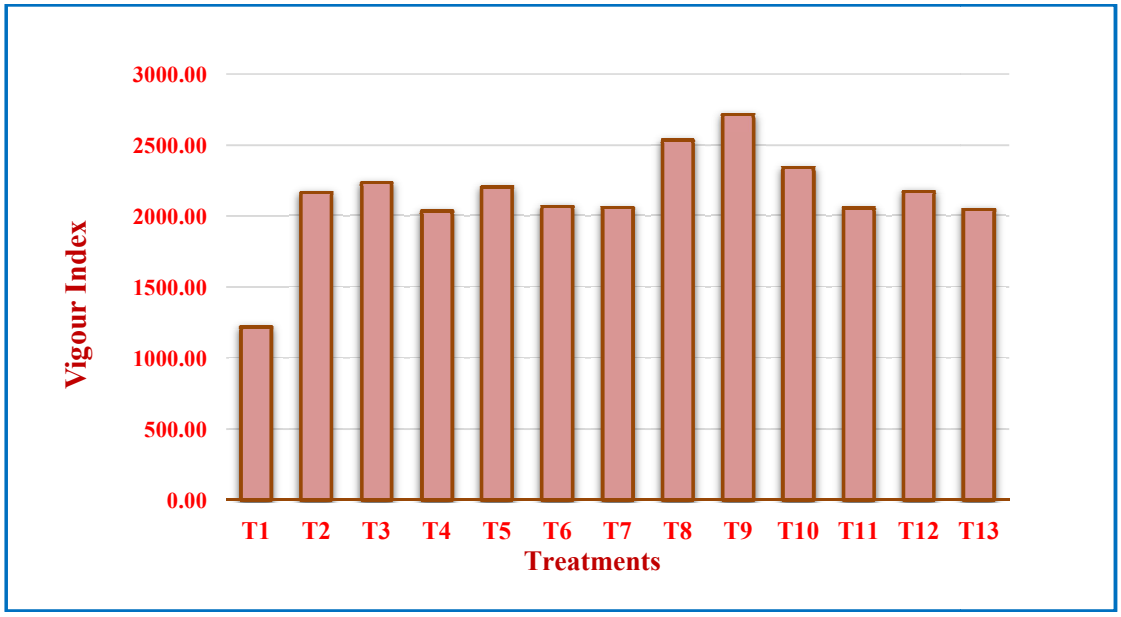

Fig. 4. Vigour Index of endophytic bacterial isolates against *Pythium aphanidermatum* in *Setaria italica* grown in seedling trays under greenhouse conditions

4. SUMMARY AND CONCLUSION

Bacterial endophytes isolated in present study have one or more biocontrol mechanisms and among 12 isolates, only five isolates were prominent in siderophore production, β-1,3-glucanases and chitinases. But all the isolates produced volatile and non-volatile compounds in both the solid and liquid medium. Moreover, some of the bacterial isolates capable of producing siderophores did not produce glucanases and chitinases and these bacterial endophytes have the ability to control fungal pathogens by occupying ecological niches in the host plant tissues similar to that of plant pathogens and thus inhibiting the fungal growth. From many scientific studies, it is known that field crops harbored efficient and competent endophytic bacterial endophytes and isolation, screening, evaluation, and molecular identification of such endophytes for plant growth-promotion and biocontrol activities, plays an important role in the sustainable model of agriculture. Further, these endophytes may be used as bioinoculants in single or consortium to achieve yield sustainability in agriculture. The biocontrol mechanisms and antagonistic abilities of bacterial endophytes can be understood completely and better by employing several approaches like the use of molecular methods, *in-vivo* expression technology, fluorescence methods to detect the endophytes in the plant can help to achieve this objective.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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