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CONTENTS

Investigating *in vitro* direct antagonistic effect of endophytic bacteria against *Alternaria brassicicola*

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ABSTRACT: Plants associated with endophytes are imbibed with beneficial growth promoting and biocontrol activity against phytopathogens are sustainable alternative to the synthetic fungicides. In the study, bacterial endophytes isolated from *Brassica* spp. were screened for antagonistic activity against *Alternaria brassicicola*, causing black spot disease in mustard. Bacterial isolate exhibiting 58.14 % mycelial inhibition rate was selected and identified based on 16S rRNA gene sequences as *Bacillus subtilis* strain and examined for further preliminary *in vitro* assays showing direct antagonism and inhibition of the pathogen. Microscopic studies showed irregularities in mycelium growth, aberrations in the spores' structure and cell integrity after interacting with bacterial cells. In the buried slide experiment, the antagonistic isolate was in physical contact with the pathogen. The results showed stunted spores, uneven germination, plasmolysis of cellular components, and distorted hyphae, restricting the proliferation of the mycelial network over a 72-hour period in inoculated soil. The potential of bacterial cells as a foliar treatment to control black spot was evaluated through a detached leaf assay every 24 h for 3 days. A significant control of necrosis progression was observed in the bacterial cell inoculated leaf with respect to control. In the control, black-brown necrotic lesions encircled by a distinct yellow halo were observed after 24 hrs, and after 72 hpi, lesions of size 13 mm with extensive chlorotic regions were observed whereas in treatment, no lesions developed after 48 h and the lesion was 1.7mm after 72 hpi. The results indicate that, with optimised augmented inoculation, bacterial cells could be employed in disease management as soil and foliar treatments in further *in vivo* experiments.

Keywords: *Alternaria brassicicola*, *Brassica* spp., Biocontrol, Endophytes

Plant pathogens significantly impact both the quality and quantity of agricultural produce, compromising food security on national and international levels (Savary *et al*., 2019). Plant pathogenic fungi are some of the most economically damaging phytopathogens. As a result, fungal diseases inflict significant harm on numerous economically vital fruits, vegetables, and food crops throughout both pre- and post-harvest phases (Dean *et al*., 2012).

In India, oilseeds account for 14.3 % of yearly gross cropped area and 8% of agricultural exports. Rapeseed-Mustard contributes 45% to the domestic edible oil production and is one of the most important oilseed crops (NITI Aayog 2024). It contributes 33.24 % of the total oil seed production according to DA&FW, Ministry of Agriculture and Farmers Welfare. Indian mustard (*Brassica juncea L. Czern & Coss.),* a member of the *Brassicaceae*, is a significant oilseed crop in India (Singh *et al*., 2022). Mustard crops are affected by biotic stress posing major challenges and leading to significant yield losses. *Alternaria* blight is the most severe threat and causes 10-70% yield loss, followed by White rust (47 %) and *Sclerotinia* rot (35%) according to the Directorate of Rapeseed-Mustard Research (DRMR). *Alternaria* blight, also referred as Black spot of oilseed, is mostly induced by *Alternaria brassicae* (Berk.) Sacc., coupled with *Alternaria brassicicola* (Schw.) Wilts. and *Alternaria raphani* Groves and Skolko. The disease symptoms include dark brown lesions/spots surrounded by concentric rings, with chlorotic yellow halo on leaves, stems and siliques. Under favourable conditions, i.e., temperatures of 20-30º C and high relative humidity (95-100%), in about 5 h, formation of black-brown conidia takes place (Nowicki *et al*., 2012).

Researchers are investigating biologically sustainable strategies to control diseases and improve yields instead of using hazardous chemicals.

Developing effective biological products in the field is essential to address both the sustainability aspect of agriculture and the concurrent growth of resistant phytopathogens (Iqbal *et al*., 2023). Chakraborty and Newton (2011) explained that pathogens may become resistant to frequently employed fungicides that are commercially available, also there is possibility that less virulent diseases in natural forests may exhibit enhanced aggressiveness in monoculture crops. Therefore, the pursuit for an effective biocontrol agent that may endure in the rhizosphere and can be closely associated with plants continues. One such approach is to develop biocontrol agents, which can be sourced from the plant system, that can easily adjust to local soil and climatic conditions without interfering with the functional niche (Fira *et al*., 2018).

Endophytes are microorganisms that establish a dynamic relationship with host plants by inhabiting the rhizosphere, phyllosphere, and endosphere (Alawiye and Babalola 2019). They promote plant growth and development by enhancing nutrient absorption, aiding host plants in overcoming biotic and abiotic stress (Santoyo *et al.*, 2016). They are diverse and multifunctional, establishing symbiotic associations without inducing biotic stress in the host plant (Gupta *et al.*, 2020). The predominant and widely utilised biocontrol agents include *Trichoderma* spp., *Penicillium* spp., *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp., and *Burkholderia* spp. (Lahlali *et al.*, (2022). Their biocontrol potential is because of the synthesis of siderophores, extracellular hydrolytic enzymes, secondary metabolites that target the cell wall and plasma membrane, and intracellular processes of pathogens (Ye *et al.*, 2019; Tran *et al.*, 2022).

The efficacy of an effective biocontrol agent is dependent on three primary mechanisms, i.e., limiting pathogen establishment through competitive exclusion, antibiosis against phytopathogens, and induction of systemic resistance in the host plant (Etesami *et al*., 2023). Therefore, the objective of our study is to screen endophytic bacteria with antagonistic potential against *Alternaria brassicicola* and to investigate the impact of the antagonist isolate on fungal morphology and spore structure. Additionally, we explored the direct mode of action of the selected isolate through soil and foliar treatments, illustrating direct antagonism and inhibition of the pathogen under *in vitro* conditions.

MATERIALS AND METHODS

Isolation of endophytic bacteria

In the study, bacterial endophytes were isolated from *Brassica* spp. harvested from the experimental farms at N.E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture & Technology, Pant Nagar, Uttarakhand, India, at 29^0 N latitude and 79.3^0 E longitude. Selected plants were thoroughly washed with running tap water and subsequently surface sterilised. Each individual plant was stripped of its leaves, and from the stalks, small pieces of 4 inches were severed aseptically 5 cm above the soil level. Each stalk piece was coated with paraffin at both ends and was surface disinfected with 70% ethanol for 60 sec, washed twice with sterile distilled water (SDW) for 1 min, further disinfected with 2% sodium hypochlorite for 1 min and finally rinsed 3 times with sterile distilled water to remove disinfectants. The distilled water used for the final rinse was checked for contaminants. Transverse sections of each piece were incubated on nutrient agar (NA) medium (HIMEDIA Laboratories, India) for 4-6 days at 24^0 C ± 2. Pure cultures of each of the bacterial isolates were obtained and preserved for further investigation

Isolation of phytopathogen and identification

Phytopathogens causing the leaf blight disease were sampled randomly from different diseased *Brassica* spp. cultivated at N.E. Borlaug Crop Research. Infected leaves were washed with running tap water and were air-dried in a sterile condition. Small sections, including the diseased spots, were cut and surface sterilised aseptically with 70% ethanol for 1 minute, 2% sodium hypochlorite for 2 minutes, and finally rinsed three times with SDW to remove disinfectants. Sections were placed on plates containing potato dextrose agar (PDA) medium supplemented with streptomycin (30 mg/L) and incubated for seven days at 24ºC ± 2. After incubation, culture was purified by single spore isolation method and pathogenicity was established

as per Koch's postulates. Pathogens were further microscopically identified based on morphological characteristics.

Screening of endophytes for anti-fungal activity against Alternaria brassicicola

In vitro antifungal activity of each isolate was examined against the phytopathogen through dual culture plate assay. Each bacterial isolate was cultured in Nutrient Broth (NB) medium for overnight at 24 ± 2 ºC. Thereafter, these bacterial isolates (OD600:0.1) were placed 2.5 cm from the fungal plug (5 mm) of a 7-day-old fungal pathogen, placed at the centre of a petri plate consisting of NA and PDA (1:1). For control, sterile NB was used. After 7 days of incubation at 24 ± 2 °C, mycelium growth (MG) on the culture plate was observed, and growth inhibition was calculated as-

$$MGI = \frac{G_c - G_I}{G_c} \times 100$$

MGI is mycelium growth percentage inhibition over control, G_C is mycelium growth inhibition in control (in cm) and G_I is mycelial growth inhibition in bacterial isolate (in cm).

Identification

Genomic DNA of selected isolate was extracted using CTAB method (Cetyl Trimethyl Ammonium Bromide). Universal primers GM3f (5¹- AGAGTT TGATCMTGGC -3¹) and GM4r (5¹- TACCTTGTTACGACTT -3¹) were used as forward and reverse primers respectively to amplify the microbial 16S ribosomal RNA gene. The PCR product was outsourced to HiMedia Laboratories for sequencing using Sanger sequencing method. The resulting gene sequence was analysed with a BLAST search against the NCBI GenBank database (https:// blast.ncbi.nlm.nih.gov). Ten sequences with the highest identity scores were selected for alignment, using the CLUSTALW multiple sequence alignment tool. A distance matrix was then generated, and a phylogenetic tree was constructed with MEGA11 software, using closely related strains.

Effect of bacterial isolate on mycelial structure and spore morphology

The effect of endophyte on spore and mycelial morphology of *A. brassicicola* was examined microscopically through dual culture assay. Selected endophyte and pathogen were cultured in proximity and incubated for 5 days at 24 ± 2 ºC. For control, NA without bacterial growth was taken. The *in vitro* interactions between the endophyte and pathogen were observed under the microscope.

Direct impact of antagonistic isolate on spore germination and growth of pathogen as soil treatment

To check the direct antagonistic impact of isolate on pathogen as soil treatment, buried slide technique was employed (Stevenson, 1956). In the experiment, about 25g soil per treatment was sieved and autoclaved three times in glass boxes. From the primary culture of bacterial isolate, 100 µl OD: 0.2 (A_{600}) was reinoculated to another flask containing 100 ml of NB and incubated at 24 ± 2ºC for 24 h at 120 rpm. Bacterial broth was centrifuged at 9000 x g for 15 min. The supernatant was removed, and the pellet was washed three times with SDW. Washed bacterial cells were resuspended in SDW, from which 10 ml of bacterial cells (1.5 x 10^6 - 10^7 CFU mL^{-1}) were inoculated in each soil box. For control, SWD was used. All the treatments were incubated for 7 days at 24 ± 2ºC. Additionally, 10 ml of fungal spore suspension (10^5-10^6 spores/ml) was prepared from a 7-day-old fungal culture plate. Culture plates were flooded with SDW supplemented with 1% Tween-20. Spores were filtered through sterile cheesecloth and after centrifugation at 9000 x g for 10 min, pellet was resuspended in SDW. Spore suspension was adjusted to 10^5-10^6 spores/ml and mixed with molten 1.5 % sterile agar. Further, 1 ml of the spore agar suspension was dispensed on a clean, sterile glass slide evenly. After the agar layer had set, glass slides were cautiously inserted vertically inside the incubated soil boxes and were further incubated at 24 ± 2ºC for 7 days. After incubation for 2, 4 and 7 days, slides were removed carefully and examined under a compound microscope. Four spore-agar coated glass slides were placed in each box. The spore germination and mycelium development were observed in control and bacterial cell inoculated soil.

Direct impact of antagonistic isolate on the growth of phytopathogen as foliar treatment

To check the direct antagonistic impact of bacterial isolate on the pathogen as foliar treatment, the detached leaf experiment was conducted. In the experiment, the fourth leaf of each *Brassica* plant was collected. Healthy leaves were collected in sample bags from the fields and were carried to the laboratory. Leaves were initially washed under running tap water and were surface sterilised under sterile conditions. After washing with sterile distilled water twice, they were air-dried under a laminar hood. From the primary bacterial broth culture, 100 µl OD:0.2 (A_{600}) was reinoculated to 50 ml of nutrient broth and incubated at 24 ± 2°C for 24 h at 120 rpm. After incubation, bacterial culture was centrifuged at 9000 x g for 15 min at 4°C, supernatant was removed, and the pellet was washed three times with SDW. The pellet was resuspended in sterile distilled water. Resuspended bacterial cells (1.5×10^6 - 10^7 CFU mL^{-1}) were sprayed on detached leaves placed in Petri plates, air dried, and were further inoculated with 10 µl of fungal spores (10^5-10^6 spores/ml). Appropriate moisture was maintained inside the plates. For control, sterile distilled water was used. Plates were incubated for 3 days at 24 ± 2°C and the spread of necrosis was observed afterwards. Three plants were used per drop inoculation.

Statistical analysis

ANOVA followed by Tukey's Honest Significant Difference (HSD) analysis was used to assess the fungal mycelium inhibition rate. The impact of time and bacterial cell on *Brassica* spp. leaves was evaluated through two-way ANOVA followed by Duncan's Multiple Range Test (DMRT). All analysis of present data is a mean of three replicates ± standard deviation at a significance level of 0.05 using the R-programming language, version 4.0 (R Core Team) using the 'Agricole' package.

RESULTS AND DISCUSSION

It has been established that the beneficial bacteria associated with plants are extremely helpful in promoting plant growth and enhancing resistance against phytopathogens. After colonising the rhizosphere, plant growth-promoting bacteria proliferate inside the host and aid in the production of phytohormones, nutrient utilisation, enzymes and siderophore production. Additionally, they improve the health of the soil by supporting diverse microorganisms and improve plant defences as high-quality bio-inoculants for stable, consistent yields and sustainable agriculture (Santoyo *et al*., 2016). Endophytic bacteria have no obligation to compete with other microorganisms for space and nourishment, as they have designated ecological niches. Therefore, they could prove to be better potent biocontrol agents.

Our study assessed that the endophytic bacteria provided insight into their antagonistic potential. A total of eighteen endophytic bacteria strains were isolated from the stem of *Brassica* spp. based on distinct morphological and cultural properties. The selected endophytic bacteria were purified and evaluated for antifungal activity. Mycelium colour, growth pattern on PDA, microscopic analysis and pathogenicity test was performed on detached leaf of *Brassica*. Muriform conidiospores, germination pattern and development of dark brown lesions on detached leaves indicate the pathogen to be *Alternaria brassicicola* (Figure 1).

All the isolates were evaluated for antagonistic activity against *A. brassicicola* using a dual culture assay. Table 1 displays mycelium growth inhibition by each isolate with respect to control. Of the 18 isolates, 15 showed an inhibitory effect on *A. brassicicola*. Isolates with the highest inhibitory percentages showed strong antagonistic activity. Subsequently, other isolates demonstrated significant inhibitory rates ranging from moderate to low. Isolate N exhibited the highest inhibition of mycelial growth at 58.14% later identified to be *Bacillus subtilis* strain BSN01 (Figure 2) indicating potential to produce antifungal compounds and was selected for further analysis (Figure 3).

Several morphological changes such as stunting, swelling, protuberances in mycelial structure, distortion, abnormal germ tube growth induced by antimicrobial compounds present in bacteria, have been previously reported by Gunji *et al.* (1983). In the study, microscopic observations illustrated the antagonism of the endophyte against phytopathogens

which include structural irregularities, uneven thinning of the hyphal structure, vacuolation, cytoplasmic exosmosis, ruptured spores in comparison to the control with prominent germ tube formation, and uniform regular conidia (Figure 4). Due to the formation of bubbles, irregular germ tube growth, and other malformations, pathogenicity is hampered. They weaken the pathogen and lessen their ability to infiltrate the host, which is an essential component of infection.

Other studies have also reported the production of antibiotics by bacteria that fight phytopathogens. Antifungal biomolecules interact with fungal cell membranes resulting in thin, uneven hyphae, irregular conidia, and plasmolysis (Ahmad *et al.*, 2023).

In order to investigate the impact of selected endophyte BSN01 as potential biocontrol, direct interaction of selected endophyte and pathogen was established through soil treatment and foliar treatment. In the buried slide technique, after 2 days of incubation, 100% spores' germination in control soil was observed, whereas in treated soil, intact stunted spores were present after the second day. On day 4, the control soil displayed a well-proliferated, even network of mycelium, while the treated soil displayed vacuolated and stunted spores. After 6 days, massive mycelial growth with new sporulation was observed in control. However, stunted, uneven germinated spores and plasmolysis of hyphae were observed in inoculated treated soil (Figure 5).

Many biocontrol agents exhibit biocontrol action because of the well-known and widespread occurrence of antibiosis. Antibiosis is the antagonism due to the toxic effects of secondary metabolites produced by one microorganism to restrict other microorganisms. The direct contact between biocontrol agent and pathogen can facilitate diffusion of these secondary metabolites and antimicrobial compounds easily. Our results corroborate with Manhas and Kaur (2016). They demonstrated that in *A. brassicicola,* loss of melanin and leakage of cellular components change extracellular conductivity and eventually the cell membrane integrity. It has also been suggested that the general availability of antibiotics in soil contributes to soil suppressiveness, a phenomenon in which plants do not exhibit disease symptoms even when pathogens and favourable conditions for disease development exist (Arseneault and Filion 2017). Garbeva *et al.,* (2011), further explained the state of bio stasis, which is when spore germination and mycelium growth is inhibited without killing the microorganism, because the microbial community produces antimicrobial compounds while using the soil nutrients, which reduces the nutrients available for pathogens in disease-suppressive soil.

In the detached leaf experiment, the development of black spot disease was evaluated in the fourth leaf of *Brassica* spp. every 24 h for three days post inoculation. The necrosis increased gradually in control and reached 13 mm at 72 hpi. Black spots started showing after 24 hpi and later developed into necrotic lesions, surrounded by a prominent yellow halo (Figure 6) and eventually a more extensive chlorotic region was observed. There is significant reduction in the growth of phytopathogen in inoculated leaves with respect to control over a period of 72 hpi ($p < 0.05$) (Table 2). The results demonstrate that the treatment was effective in suppressing the disease proliferation over time and that foliar spray of endophytic isolate BSN01 could be considered to evaluate *A. brassicicola* antagonism and resistance. Abdel-Gaied *et al*. (2020), demonstrated better disease management of soft rot in potatoes by biocontrol agents through foliar spray than the soil treatment.

A. brassicicola penetrates the host foliar tissue and a detached leaf assay could be a reliable way to evaluate the symptoms of dark leaf spots, the inhibition of phytopathogen due to competition, antibiosis, and induction of systemic resistance in hosts (Etesami *et al.,* 2023) In order to colonise the hosts, fungi have evolved a wide range of strategies, such as intense spore production, appressorium development, production of cell wall degrading enzymes, and the inhibition of plant defence responses (Lo Presti *et al*., 2015).

In response to these strategies of fungi, biocontrol agents boost plant defences, facilitate parasitism and antibiosis, and compete with phytopathogens for space and resources (Köhl *et al*., 2019). They have

the potential to restrict infection by directly inhibiting the proliferation of pathogen through synthesis of low molecular weight peptides, polyketides, volatile compounds, non-ribosomal peptides, and other different classes of metabolites with antifungal activities (Hassan *et al*., 2022).

Advantages to consider foliar treatment as a direct mode of action include the positive impact of biological control agents on the phyllosphere microbial community. Qin *et al*., (2019) evaluated tobacco wildfire disease where they effectively suppressed the disease, significantly altered microbial diversity, its composition, and interactions in the phyllosphere and it was observed that higher microbial diversity was linked to lower disease severity. In another study, the biocontrol agents, especially when combined with magnesium, can help manage both disease and antibiotic resistance genes dynamics in the phyllosphere, offering a more environmentally friendly alternative to traditional antibiotics (Zhi *et al*., 2024). Generally, it is presumed that extensive induction of biocontrol agents is required for their sustenance in uncontrolled conditions. Also, the secondary metabolites produced by them vary under influence of other abiotic factors. *Bacillus* species have been used extensively as plant biocontrol agent. Blake *et al.* (2021) extensively reported possible strategies adopted by *B. subtilis* to protect plants i.e. directly through antibiosis, by triggering induce systemic resistance in plants, by production of fungal cell wall degrading exoenzymes and spores' resistant to probable biotic and abiotic stresses makes it a convenient agriculture input with desirable formulation.

Therefore, a multidisciplinary approach may be used to provide better perspective and formulations for effective biotic stress management. The quest to understand the antagonistic potential of biocontrol agents, and the mechanisms of antifungal metabolites in natural settings, especially in soil environments, continues to pose a challenge. Through augmented soil management techniques or optimised foliar application of biocontrol agents, the plant defence system can be enhanced.

Table 1: Screening of bacterial isolates for antifungal activity by dual medium assay

Isolate	Mycelium Growth (cm)	Mycelium growth inhibition (%)
Control	5.27 ± 0.31 *ab*	-
P1.1	4.47 ± 0.12 *cd*	15.08 ± 0.30 *h*
P1.2	2.73 ± 0.12 *fg*	48.05 ± 0.43 *c*
P1.3	3.00 ± 0.20 *ef*	42.96 ± 0.98 *d*
P1.4	4.93 ± 0.12 *bc*	6.04 ± 0.15 *k*
P2.1	5.53 ± 0.12 *a*	-5.28 ± 0.05 *n*
P2.2	4.80 ± 0.20 *bc*	8.71 ± 0.09 *j*
P2.3	4.20 ± 0.00 *d*	20.08 ± 0.41 *g*
P3.1	5.60 ± 0.00 *a*	-6.56 ± 0.07 *n*
P4.1	2.40 ± 0.00 *g*	54.33 ± 1.13 *b*
P4.2	4.20 ± 0.20 *d*	20.17 ± 0.35 *g*
P5.1	4.60 ± 0.35 *cd*	12.70 ± 0.09 *i*
P5.2	5.33 ± 0.12 *ab*	-1.42 ± 0.02 *m*
P7.2	4.93 ± 0.12 *bc*	6.04 ± 0.15 *k*
P7.3	5.20 ± 0.00 *ab*	1.05 ± 0.04 *l*
P27	4.87 ± 0.23 *bc*	7.42 ± 0.23 *jk*
P34	3.13 ± 0.12 *ef*	40.30 ± 0.30 *e*
P35	3.53 ± 0.31 *e*	32.97 ± 0.39 *f*
N	2.20 ± 0.00 *g*	58.14 ± 1.03 *a*
C.D.	0.53	1.48
C.V.	4.078	2.42

Selected isolates were cultured with pathogen. Reported data values are mean ± s.d. Experiment was performed three times. Error bar represents approx. 95% confidence level.

CONCLUSION

The study provides an insight on endophytic *Bacillus subtilis* BSN01 exhibiting antifungal properties that suppress the proliferation of *Alternaria brassicicola* upon direct contact *in vitro* conditions, it alters the spores structure and membrane integrity. Antibiosis displayed through direct contact assay contributes to the evidence supporting the possible application

Fig. 1: Isolation of phytopathogen: (A) Infected *Brassica* leaf sampled from the field (B) Isolated spores of *Alternaria spp.* (C) Reinoculation of isolated spores to *Brassica juncea* (D) Development of black necrosis, completing the Koch's postulates.

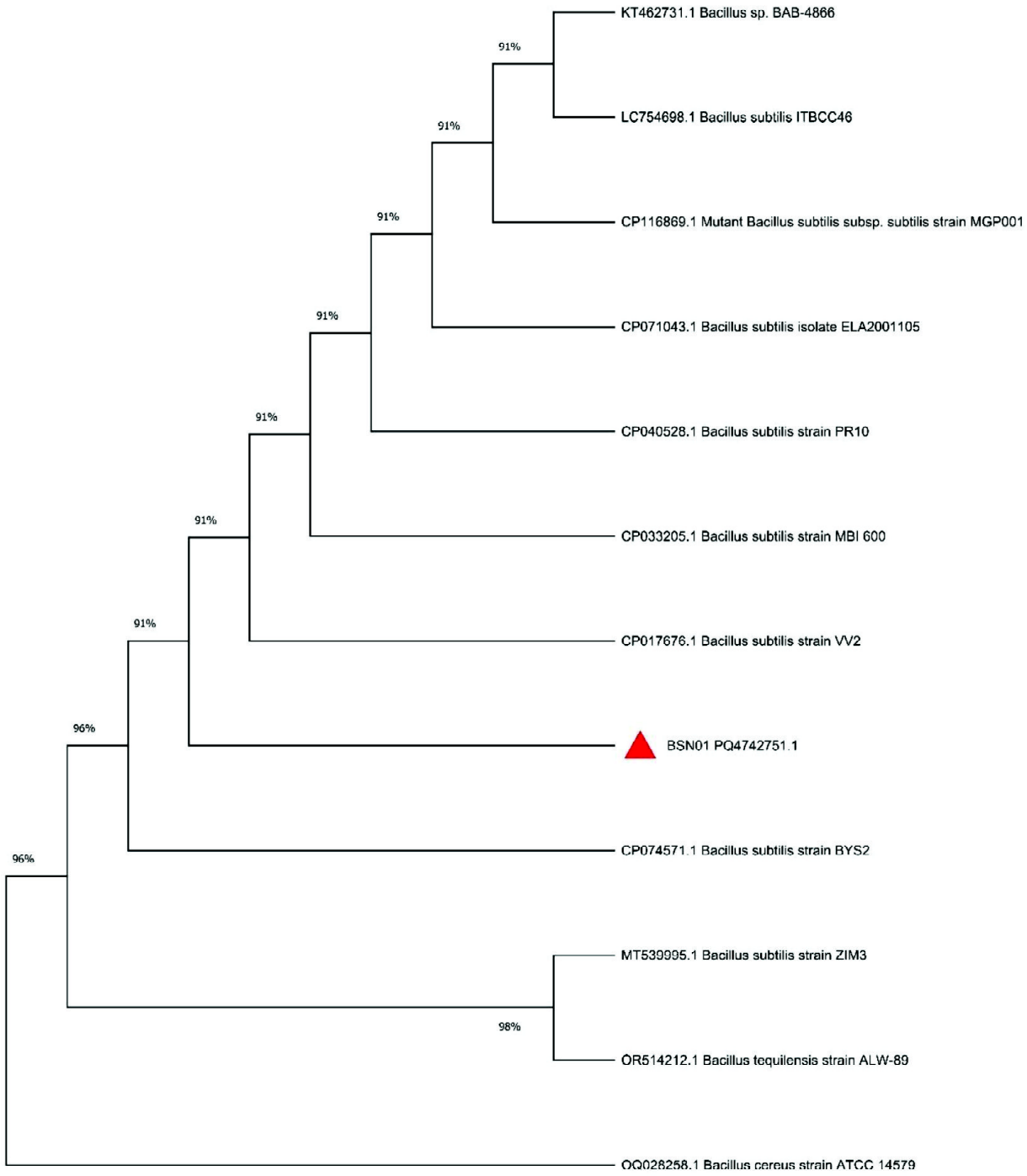

Fig. 2: Phylogenetic tree for 16S rRNA gene of bacterial isolates using Clustal W multiple sequence alignment tool. Evolutionary relationships were inferred using the Maximum Likelihood method based on the Kimura 2-parameter model. The final tree, a consensus obtained from 1000 bootstrap replicates, reflects the evolutionary history of the taxa analysed. Branches reproduced in fewer than 50% of the bootstrap replicates were collapsed.

of endophytic bacteria against the pathogen. The parameters optimised in our investigation will facilitate comparisons of assay efficiencies and will subsequently be employed in further *in vivo* experiments.

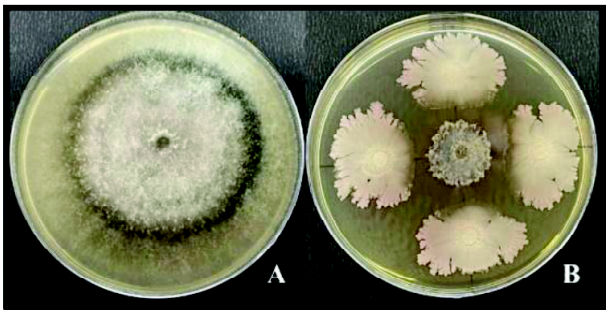

Fig. 3: Dual culture assay showing in vitro mycelial inhibition of *Alternaria brassicicola* by endophytic bacteria *Bacillus subtilis* BSN01

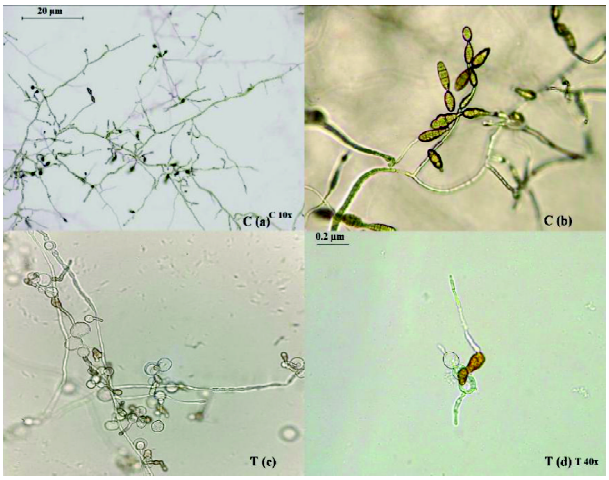

Fig. 4: Effects of isolate N on mycelial growth and morphology of *Alternaria brassicicola*: (a) Microscopic image showing regular mycelium growth and conidia at 10x (Control) (b) Intact uniform fungal spores (Control) (c) Vacuolated uneven fungal mycelium co-cultured with bacterial cells showing exosmosis of cytoplasm leading to thinning of hyphae and swollen germ tube ends (d) Formation of bubbles at germination point on conidia

In detached leaf assay, necrotic spots were observed and size was measured (mm) every 24h for 3 days. Reported data values are mean ± s.d., means with same letters within treatments are not significantly different ($p \leq 0.05$) according to two-way ANOVA followed by Duncan's Multiple Range Test (DMRT).

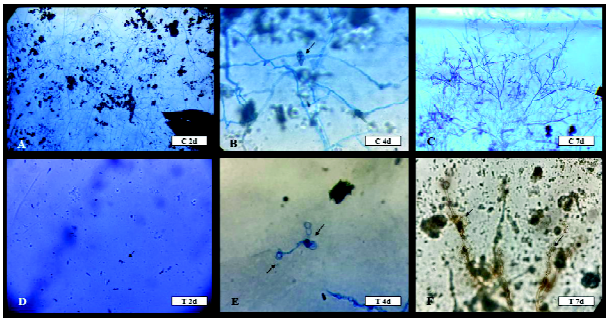

Fig. 5: Effect of BSN01 (on germination of *A. brassicicola* spores as a soil treatment (A) Massive mycelium growth in control after 2 dpi (B) Intact uniform spore germination in control (C) New sporulation and mycelial network after 7 dpi in control (D) isolate N inoculated soil with inhibited spore germination (E) Vacuolisation of germ tube (F) plasmolysis of hyphae after 7 dpi

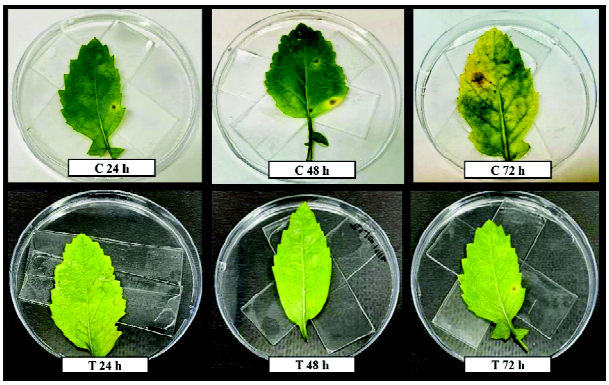

Fig. 6: Detached leaf assay showing direct impact of antagonistic BSN01 (on the growth of phytopathogen as foliar treatment for 3 dpi; C (Control, uninoculated), T (Treatment, Inoculated)

Table 2: Direct impact of antagonistic isolate N on the growth of phytopathogen as foliar treatment.

TREATMENTS	TIME (dpi)			MEAN ± SD
	24 h	48 h	72 h	
CONTROL -Uninoculated	3.67 ± 0.58 *c*	6.10 ± 0.360 *b*	13.00 ± 1.00 *a*	7.59 ± 4.24 *a*
TREATMENT-Inoculated bacterial N cells	0.33 ± 0.58 *e*	0.67 ± 0.577 *de*	1.70 ± 0.26 *d*	0.90 ± 0.75 *b*
	2.00 ± 0.77 *c*	3.38 ± 1.23 *b*	7.35 ± 2.54 *a*	
C.D.	TREATMENT	0.6219		
	TIME	0.9326		
	INTERACTION	1.0772		

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