



Soybean Bacterial Endophytes against Anthracnose Disease Collected from Karnataka State: An *in-vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Endophytes trigger various defence mechanisms within their host plants, engaging primary and secondary protective pathways. This investigation primarily aimed to isolate bacterial endophytes from diverse agroecological regions in Karnataka. Subsequently, these endophytes were assessed for their inhibition against *Colletotrichum truncatum* using the *in-vitro* streak plate technique. A total of 43 bacteria isolated from soybean plants and key endophytes showing the inhibition against *C. truncatum* were in different zones namely, DHW-9(87%), BID-2(85%), BID-13(85%), BID-14(82.50%), DHW-15(80%), BID-15(75%),and BID-16(75%) exhibited notable efficacy against *C.*

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truncatum in decreasing order. Among these, the DHW-9 (*Stenotrophomonas maltophilia* strain P4-32) bacterial endophytes isolated from the North Transition Zone (Dharwad) were highly effective against the pathogen, possibly due to employing many direct and indirect mechanisms. Furthermore, the inhibition potential of the bacterial endophytes varies with and within the place of agroecological zones. In conclusion, it has been observed that the bacterial endophyte DHW-9 inhibited the progression of anthracnose disease caused by *C. truncatum* in controlled *in vitro*. Hence, it is imperative to conduct additional experiments, including pot and field studies, to explore its potential to enhance the growth and yield of soybean plants.

Keywords: Soybean; bacterial endophyte; anthracnose; *Collectotrichum truncatum*; biocontrol; agroecological zones; growth inhibition percentage.

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a valuable crop used for both human and animal consumption due to its notable protein content of 40% and oil content of 20% by Abdelghany et al. [1]. In agriculture, it is crucial to have disease-free seeds for planting. Soybean is susceptible to various stress factors, both from living organisms and environmental conditions, at different stages of its growth, from seed germination to full maturity. Diseases, in particular, lead to substantial losses in yield each year by Rajput et al. [2]. Maintaining the health of soybean plants is vital for a profitable harvest. One of the main challenges in increasing soybean productivity is its vulnerability to different fungal diseases like anthracnose, charcoal rot, *Rhizoctonia* root rot, yellow mosaic virus, and rust. These diseases significantly impact soybean output, with anthracnose being a growing global concern by Singh et al. [3].

The *C. truncatum* is prevalent in almost all soybean-growing regions worldwide. It manifests symptoms on the stem, leaves, pods, and seeds. Warm temperatures (30-35°C) and rain mist, which provide moisture for 12 hours or more, create favourable conditions for the disease by Rajput et al. [2] & Nataraj et al. [4]. Anthracnose can lead to substantial losses, especially during the rainy season. This disease has caused yield losses in several countries across Asia, Europe, and South and North America, reaching up to 50% in Thailand and 100% in India by Jones et al. [5]. In India, soybean anthracnose caused 16-25% yield loss by Nataraj et al. [6]. Recently, Rajput et al. [7] revealed that a 1% increase in soybean anthracnose disease severity reduced the yield of 115 kg/ha of soybean grain. Various disease management techniques mitigate anthracnose occurrences, including cultural practices, crop rotation, chemical fungicides, soil solarization by Adrees et al. [8], and biological

control. However, the unregulated application of chemical fungicides has detrimental effects on the environment and presents hazards to human health and the native soil microorganisms Rani et al. [9]. Thus, sustainable agricultural practices for managing *C. truncatum* disease are imperative. Alternative methods for anthracnose control involve using biocontrol agents like *Bacillus* spp. by Sabate et al. [10] and *Pseudomonas* spp. by Moin et al. [11] and protective measures by Bernardo-Mazariegos et al. [12]. Bioagents with biocontrol properties are emerging as a potentially eco-friendly alternative to harmful conventional fungicides.

Endophytes are effective biological control agents in sustainable agriculture by Dalal et al. [13]. Some endophytes interact directly with the host plant, making them more efficient against plant pathogens and act as biocontrol agents in sustainable agricultural production, Brunda et al. [14]. According to Chauhan et al. [15], endophytes can trigger defence responses during pathogen attacks and influence host plants' physiological traits. Although the exact nature of the relationship between plants and endophytes is not fully understood, it has been observed that specific isolates benefit their hosts by producing new chemicals and antifungal substances, thereby reducing disease progression. Furthermore, endophytes have demonstrated their ability to support plants in absorbing and utilizing nutrients by providing phytohormones, molecules, enzymes, and siderophores, an antimicrobial agent. Moreover, endophytes confer additional advantages to plants, including nitrogen fixation, increased tolerance to high temperatures and drought, adaptation to osmotic stress conditions, and other benefits by Khan and Doty [16]. In the present study, we studied whether the bacterial endophytes isolated from the different agroecological zones showed differential inhibition potential against soybean anthracnose

pathogen *C. truncatum*. The soil and climatic conditions largely influence the bacterial endophyte composition of the plant. Therefore, we hypothesized that the bacterial endophytes isolated from the different agroecological zones have other and will have differential inhibition potentials.

2. MATERIALS AND METHODS

2.1 *Colletotrichum truncatum* Inoculum Preparation

The culture of *C. truncatum* was isolated on potato dextrose agar (PDA) plates composed of Potato extracts (200 g), Agar (20 g), Dextrose (20 g), and Distilled water (1000 ml). To maintain pure cultures of *C. truncatum*, a small portion from the original culture was transferred to a new PDA plate through successive sub-culturing. These plates were then incubated at a temperature of $27 \pm 10^\circ\text{C}$ for 15 days.

2.2 Isolation of Soybean Bacterial Endophytes

The bacterial endophytes were isolated from the stem and root of soybean plants cultivated in diverse agroecological regions within Karnataka (KA). In total, 43 bacterial endophytes were collected from various zones across Karnataka (refer to Table 1), encompassing the North East Transition Zone (Bidar) and North Transition Zone (Dharwad). Since morphological characteristics play a crucial role in taxonomic classification, attributes such as texture, margin, form, elevation, and colour of the bacteria were examined (Table 2). To isolate endophytic bacteria, soybean plants were collected during the flowering stage. The fresh root and shoot samples underwent a thorough washing with running tap water to eliminate any adhering soil particles, then immersed in 70% ethanol for one minute. The plant samples were then treated with 1% sodium hypochlorite for five minutes and then rinsed in 70% ethanol for another one minute or finally, the samples underwent three rinses with sterile distilled water, each lasting for two minutes, before being left to air dry. Post-pretreatment, the samples were homogenized in sterile distilled water using a sterilized mortar and pestle. The root and shoot samples were crushed separately using two distinct mortar and

pestle sets. About 1 ml of the crushed sample was serially diluted, and 1 ml (equivalent to 1000 microlitres) of aliquots from dilutions ranging between 10^{-1} to 10^{-6} were evenly spread onto King's B medium plates (Composition: Protease peptone-20.0 g, K_2HPO_4 (anhydrous) -1.5 g, Glycerol- 10.0 ml, Agar-18.0 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -1.5 g, pH- 7.2) using a sterilized glass L-rod. This plating process was carried out in triplicates, and all plates were then kept in an incubator at 28°C for 48 hours. Following incubation, distinct bacterial colonies displaying different color of colony, texture, consistency, and size variations were selected and streaked onto nutrient agar medium using the streak plate method. These plates were once again incubated at 28°C for 48 hours. A subset of representative isolates was chosen from each sample based on the differences in cultural morphology for further investigations.

2.3 Assessing Growth Inhibition Percentage of the *C. truncatum* by the Potential Bacterial Endophytes

The antagonistic potential of 42 bacterial endophytes against *C. truncatum* was assessed using the dual culture method, following the procedure outlined by Dennis and Webster [17]. On Soybean Casein Digest Agar (Tryptone Soya Agar) (Composition- Tryptone- 15gm/lit, soya peptone- 5.0gm/lit, sodium chloride- 5.0gm/lit, agar- 15gm/lit) medium. Petri dishes were filled with approximately 20ml of Soybean Casein Digest Agar and allowed to solidify. A sterile cork borer was used to cut a 5 mm agar disc from the fungal pathogen, which was then placed near the periphery of the SCA plate. The bacterial isolate was aseptically streaked at the centre. Plates without an antagonist were used as controls for the pathogen. The incubation occurred at $25 \pm 10^\circ\text{C}$ for seven days, and two replications were maintained for each treatment. The magnitude of antagonistic activity demonstrated by the biocontrol agents upon contact with the pathogen was assessed by recording the colony diameters in two directions, and an average was in both the dual culture plate and the control plate. The calculation of the growth inhibition percentage followed the formula outlined by Vincent [18]:

$$I = (C - T)/C \times 100$$

Here,

I represent the percentage inhibition of mycelium growth, C denotes the linear growth of the fungus in the control group (in centimetres), and T the linear growth of the fungus in the treatment group (in centimetres).

2.4 Molecular Characterization of Endophytic Bacteria

We have identified distinct bacterial endophytes using 16s rRNA sequencing due to their ubiquitous presence, conserved segment, and appropriate size (1500 bp) suitable for computational analysis. Typically, bacterial DNA is extracted using the CTAB (cetyltrimethylammonium bromide) technique Rajput et al. [19]. The purity and amount of genomic DNA were estimated in the nanodrop. The polymerase chain reaction was used to amplify the 16S rRNA gene, employing universal primers: 41f (5'-GCTCAAGATTGAACGCTGGCG-3') and 1488r (5'-GTTACCTTGTTACGACTTCADD-3'), following the method outlined by Maatallah et al. [20]. A 30 µl PCR reaction mixture consisting of Nuclease free water of PCR grade nuclease - 9.0 µl, 2 x multiplex PCR master mix - 15.0 µl, 1.5 µl of Forward primer, 1.5 µl of Reverse primer, BSA- 1.5µl, Genomic DNA 1.5 µl was prepared. The samples were amplified using a Polymerase Chain Reaction (PCR) technique that included 5 minutes of initial denaturation at 94 °C, 30 seconds of annealing at 58 °C, and 1 minute and 30 seconds of elongation at 72 °C. This cycle was repeated 34 times before stopping at 4°C. The PCR products were then separated on a 1% agarose gel at 90 volts. Using the BLAST tool, the resultant sequences were matched to the GenBank database, and a multiple sequence alignment was performed using CLUSTAL W. Finally, the bacterial endophytes' DNA sequences were submitted to the NCBI database to obtain accession numbers.

2.5 Data Analysis

The data was statistically analyzed using R Studio. Graphs were prepared using GraphPad Prism software (San Diego, CA, USA).

3. RESULTS

3.1 Diversity of Soybean Bacterial Endophytes

We focused on the antagonistic bacterial endophytes isolated from diverse agroecological

zones within Karnataka. A total of 43 bacterial endophytes were isolated from the stem and root tissue of soybean plants from different locations throughout Karnataka. The diversity of bacterial endophytes was calculated using the Shannon-Weiner Diversity Index and Simpson Index. According to our findings, the agroecological zones had a significant ($p < 0.001$) effect on endophytic bacterial diversity. The Shannon diversity index for root and shoot was significantly higher in the north transition zone region (1.56, 1.62). Similarly, The Simpsons- weiner diversity index for root and shoot was significantly higher in the north transition zone region (0.42, 0.47) (Table 1).

3.2 Identification of Potential Endophytic Bacterial Through Streak Plate Technique

The endophytic bacterial isolates collected from different agroecological zones had shown differential inhibition potential. Moreover, within the same region, the inhibition potential differed for the different bacterial isolates against *C. truncatum*. Among 20 bacterial endophytes isolated from the north transition zone region, maximum antifungal activity against *C. truncatum*. 85.00 % was obtained from BID-2 and BID-13, which was not showing significant with isolate BID-14(82.50%), BID-15(75.00 %) and BID-16(75.00 %) and showing significant higher than remaining isolate (Fig. 1). Among 23 bacterial endophytes isolated from the northeast transition zone region, maximum antifungal activity against *C. truncatum* 87.00 % was obtained from DHW-9, which was not showing significant with isolate DHW-15 (80.00%), DHW-18 (67.00 %) and DHW-23 (67.00 %) and showing significantly higher than remaining isolate (Fig. 2).

3.3 Morphological Characteristics of Bacterial Endophytes

The morphological characteristics of bacterial endophytes were detected, such as texture- circular, irregular, margin- entire, undulate, elevation-flat, convex, elevated, and colour- yellow, white, and so on (Table 2). The North Transition Zone showed 55% and 45% circular and elevated forms, respectively. Additionally, all the endophytes had an entire shape margin and had elevation raised 35 %,

convex 25%, flat 25 % and elevated 15 %. Similarly, the colours were off-white 35%, white 30%, dark-off white 15%, yellow 15%, and transparent 5%. In the North East Transition Zone, we found circular, thread and irregular forms were 52.17 %, 4.34 %, and 43.47 %, respectively. Similarly, the bacterial margin was of entire 42.17 %, undulate 39.13 %, bunch 4.34 %, and lobate 4.34 % type. Moreover, the elevation was raised by 43.47 %, flat 30.43 %, and convex 26.08 % type. The bacterial colours were off-white type 30.43 %, white 21.73 %, transparent 21.73 %, yellow 13.04 %, pink 8.69 % and light pink 4.34 % (Table 2).

3.4 Endophytic Bacteria Molecular Characterization

The strains DHW-9 and BID-2 were characterized through 16S rRNA gene sequencing. Subsequently, the obtained amplicons were sequenced, and the resulting nucleotide sequences were deposited in GenBank (NCBI accession codes OR616566 and OR616569). Analysis of the nucleotide sequences, including homology and BLAST analysis, confirmed that DHW-9 corresponds to *Stenotrophomonas maltophilia* strain P4-32, while BID-2 corresponds to *Alcaligenes aquatili* strain BC5 (Fig. 4).

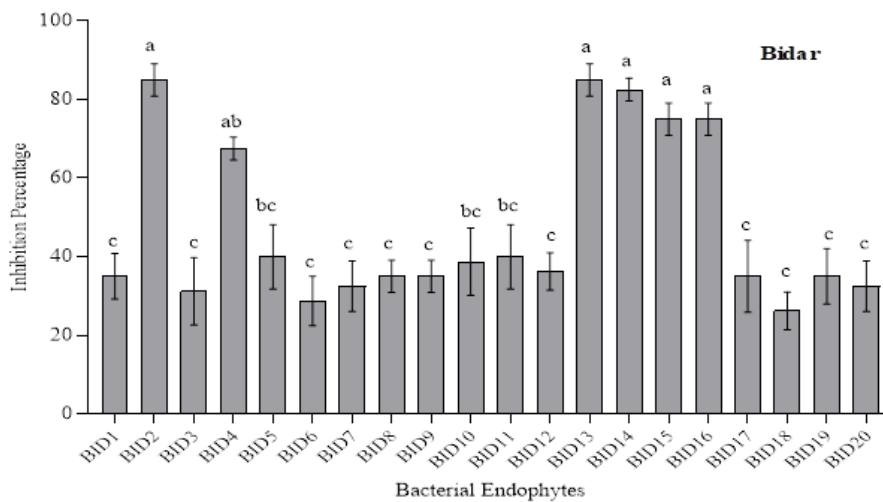


Fig. 1. *In-vitro* evaluation for the antifungal activity of bacterial endophytes against *C. truncatum* of Bidar

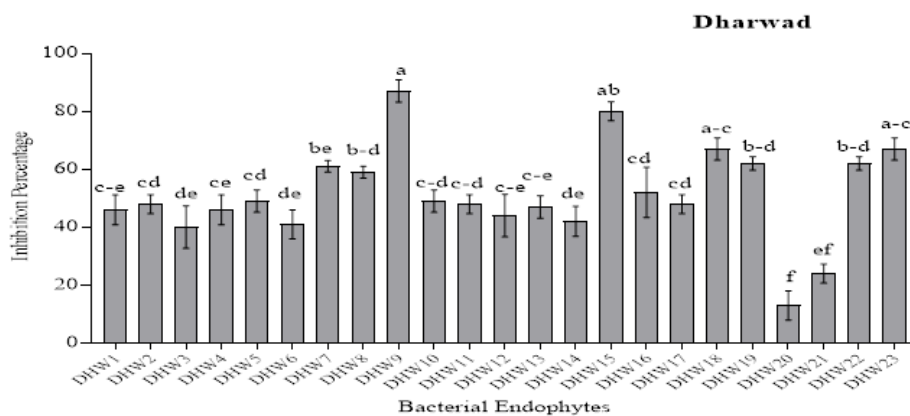


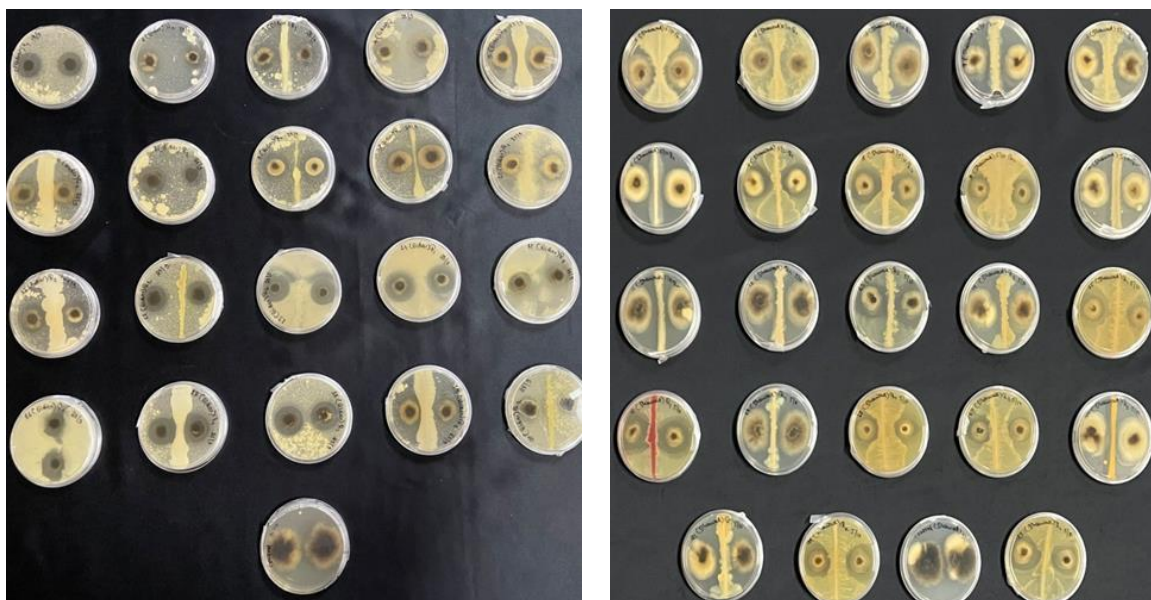
Fig. 2. *In-vitro* evaluation for the antifungal activity of bacterial endophytes against *C. truncatum* of Dharwad

Table 1. The diversity index of soybean growing agroecological region by Shannon-Weiner diversity and Simpson's index method

Region	Shannon Weiner Diversity		Simpsons index	
	Shoot	Root	Shoot	Root
North Transition Zone	1.56 ^a	1.62 ^a	0.42 ^a	0.47 ^a
North East Transition Zone	1.28 ^b	1.45 ^b	0.31 ^b	0.30 ^b

Table 2. Morphological characteristics of bacterial endophytes isolated from different agroecological zones of Karnataka state

Region	Morphology	Shape	Percentage	Region	Morphology	Shape	Percentage
North East-Zone	Form	Circular	55	North East Transition Zone	Form	Circular	52.17
		Elevated	45			Thread	4.34
		Entire	100			Irregular	43.47
	Margin	Raised	35		Margin	Entire	52.17
		Convex	25			Bunch	4.34
		Flat	25			Undulate	39.13
	Elevation	Elevated	15		Elevation	Lobate	4.34
		White	30			Convex	26.08
		Off-white	35			Flat	30.43
	Colour	Transparent	5		Colour	Raised	43.47
		Dark off-white	15			Off-white	30.43
		Yellow	15			Pink	8.69
						White	21.73
						Transparent	21.73
			Yellow	13.04			
			Light pink	4.34			



Bidar

Dharwad

Fig. 3. In-vitro evaluation for the antifungal activity of bacterial endophytes against *C. truncatum*

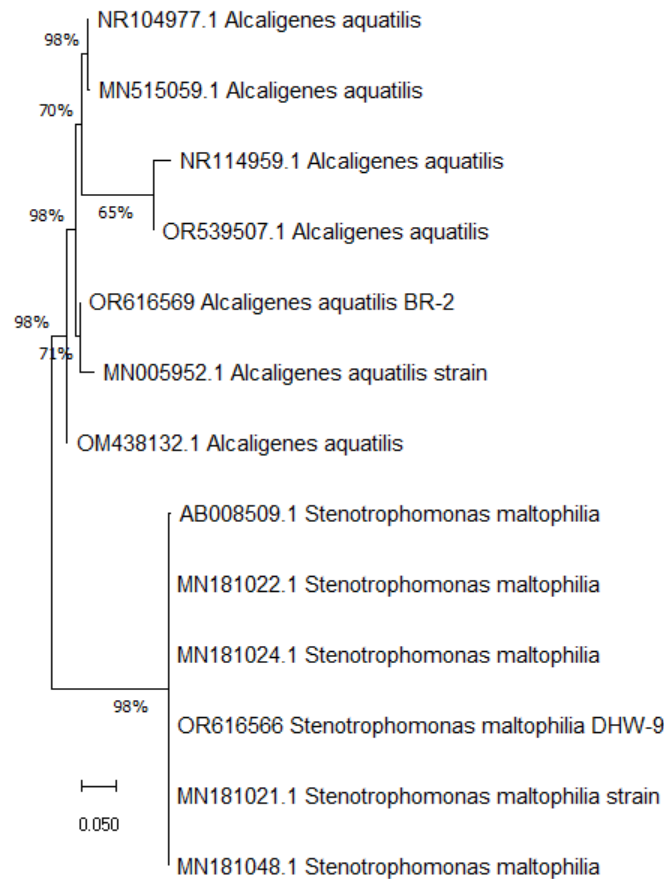


Fig. 4. The phylogenetic relationships by neighbor joining method of the *Stenotrophomonas maltophilia* strain P4-32 (DHW-9) and *Alcaligenes aquatilis* strain BC5 (BID-2) based on 16SrRNA gene analysis. Evolutionary distances were calculated using the Mega-11 software

4. DISCUSSION

Anthraxnose, caused by various lineages of the hemibiotrophic fungus *C. truncatum*, poses a significant threat to soybean productivity. While anthracnose losses have been overlooked, they could affect up to 50% of grain production. The robust survival of *C. truncatum* has impeded the effective management of this disease, the absence of suitable resistant soybean varieties, and the persistence of conventional fungicides in seed treatments. Therefore, there is a pressing need to explore alternative, environmentally sound approaches for managing anthracnose, including the potential use of biocontrol agents. Considering these challenges and the ecological aspect, our study aimed to identify a potent biocontrol agent against *C. truncatum* and assess its effectiveness in disease management and its capacity to promote plant growth. In our research, 43 bacterial endophytes were isolated

from the stem and root tissue of soybean plants from different locations throughout Karnataka, from the north transition zone and northeast transition zone region. The diversity index for root and shoot was significantly higher in the north transition zone region. Our results indicated that soybean roots have more diverse endophytes rather than the aerial part (shoot) of the soybean plant. Almida et al. [21] observed variations in bacterial populations across different plant tissues and cultivars in all of their examined field sites. Besides plant tissues and genotypes, environmental factors like temperature and soil type were identified as potential influencers of the endophytic bacteria. In a separate investigation by Egamberdiyeva and Hoflich [22], focused on plant growth-promoting bacteria isolated from wheat grown in diverse soil and temperature conditions, it was found that *Mycobacterium* sp., *P. fluorescens*, and *P. agglomerans* obtained from a semi-

continental climate notably enhanced the root and shoot growth of winter wheat at 16°C as opposed to 26°C in loamy sand. In their 2022 study, Sharma et al. [23] selected *Cordia dichotoma*, a medicinal plant indigenous to India's Jammu region, to isolate and characterize culturable endophytic bacteria and assess their antibacterial attributes. Established surface sterilization protocols were employed to isolate 33 visibly distinct endophytic bacteria from various parts of the plant, including the roots, stem, and leaves. Notably, the diversity index was considerably higher for the roots compared to the stem and leaf tissues within these plant parts.

In their 2021 estimation, Pacheco et al. [24] isolated a total of 315 bacterial endophytes from leaves, roots, and stems. These endophytes were subsequently characterized through 16S ribosomal gene sequencing. Among the identified genera, *Stenotrophomonas*, *Bacillus*, and *Pseudomonas* were the most prevalent. Each examined tissue revealed distinct bacterial species found throughout the plant, including *S. maltophilia*, *Paenibacillus castaneae*, and *P. fluorescens*. While there were no significant disparities in biodiversity indices, it was observed that root tissues had the highest abundance of bacterial endophytes.

These findings showed that bacterial endophytes isolated from healthy soybean tissues from shoots and roots suppressed the mean growth of the soybean plant disease *C. truncatum*. Seven of the 43 bacterial endophytes effectively prevented the growth of *C. truncatum*. The streak plate examine showed that DHW-9 (*Stenotrophomonas maltophilia* strain P4-32) was the most effective against *C. truncatum* of the seven endophytes tested. Furthermore, bacterial endophytes produce cell wall-degrading enzymes such as cellulase, 1, 3-glucanase, and chitinase, which are responsible for their mycoparasitic abilities Singh and Gaur. [25]. Likewise, Feng et al. [26] found that the endophytic bacteria *Bacillus siamensis* WB1 demonstrated greater efficacy against fourteen distinct plant diseases. It also notably the germination of spores related to a disease that affects walnut trees, known as walnut anthracnose (*C. acutatum*). *Bacillus siamensis* WB1 induces siderophores and indole-3-acetic acid, as well as extremely efficient chemicals such as antifungal lipopeptides and extracellular hydrolytic enzymes. In a 2013 study, Abraham et al. [27] identified six bacterial endophytes from

different parts of *Hevea brasiliensis* plants, including petioles, leaves, and root tissues. Notably, the bacterial endophyte EIL-2 exhibits a 62-64% inhibitory effect on *Phytophthora Meadii* disease. EIL-2 was identified as *Alcaligenes* sp., while the other isolates were classified as *Pseudomonas* sp. In Soumyamol et al.'s [28] research from 2023, these findings validate that the five identified bacterial isolates from the roots and leaves of rubber plants. Among these, *Stenotrophomonas* sp., when subjected to dual culture testing, demonstrated an impressive 62 percent inhibition of radial growth, which exhibits growth-promoting traits and holds promise as a biocontrol agent against *Colletotrichum* sp., a highly destructive pathogen affecting rubber crops. These bacterial endophytes were identified using a combination of 16S rRNA gene sequencing and biochemical study. Siderophore generation, cellulase production, citrate utilization, and oxidase production were all positive features in all five isolates. Similarly, the genus *Stenotrophomonas* has been employed as a potential microbial agent for biocontrol against various plant diseases that cause damage to commercial crops, as identified by Berg et al. [29] in 2002. As per the findings of Ryan et al. [30] in 2009, *Stenotrophomonas* sp. has shown antagonistic effects on plant diseases, along with exhibiting properties that promote plant growth. Certain species within the *Stenotrophomonas* genus, notably *Stenotrophomonas rhizophila* and *Stenotrophomonas maltophilia*, have shown favourable interactions with plants. This genus is gaining prominence as a source of biofertilizers. It is recognized for its production of osmoprotective compounds that play pivotal roles in cellular processes like DNA replication and cellular metabolism and serve as potent enzyme stabilizers Kumar et al. [31]. In their recent study, Ray and researchers [32] aimed to employ heat-treated *S. rolfsii* to induce the production of antifungal chemicals in the endophytic bacterium *Alcaligenes faecalis* BHU12. *In vitro* experiments revealed that the cell-free supernatant obtained from cultivating BHU 12 in the presence of freeze-crushed and autoclaved *S. rolfsii* substantially led to hyphal degeneration of *S. rolfsii* and suppression of sclerotial germination. Likewise, Fairman et al. [32] discovered that the *Stenotrophomonas maltophilia* strain UPMKH2 effectively suppressed the development of rice blast disease and subsequently enhanced crop yield and its potential as a viable biocontrol agent [33].

5. CONCLUSION

This study shows strong evidence supporting the effectiveness of bacterial endophytes in controlling *C. truncatum* infection, which leads to anthracnose disease in soybean plants. The involvement of endophytes in stimulating plant defences and enhancing the growth of soybean plants. Our findings highlight the potential of DHW-9, followed by BID-2, BID-13, DHW-15, BID-14, BID-15, and BID-16, in inhibiting pathogen growth, possibly through the production of antifungal compounds and CWD enzymes. Further research should explore their effectiveness in real-world farming conditions, their impact on plant growth, and the specific mechanisms by which they counteract pathogens. This will improve our comprehension and utilization of endophytes as highly efficient agents in managing plant diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abdelghany AM, Zhang S, Azam M, Shaibu AS, Feng Y, Qi J, Sun, J. Exploring the phenotypic stability of soybean seed compositions using multi-trait stability index approach. *Agronomy*. 2021;11(11):2200.
2. Rajput LS, Nataraj V, Kumar S, Amrate PK, Jahagirdar S, Huilgol SN, Khandekar, N. WAASB index revealed stable resistance sources for soybean anthracnose in India. *The Journal of Agricultural Science*. 2021;159(9-10):710-720.
3. Singh P, Chatterjee A, Rajput LS, Kumar S, Nataraj V, Bhatia V, Prakash, S. Biospeckle-based sensor for characterization of charcoal rot (*Macrophomina phaseolina* (Tassi) Goid) disease in soybean (*Glycine Max* (L.) Merr.) crop. *IEEE Access*. 2021;9:31562-31574.
4. Nataraj V, Rajput LS, Shivakumar M, Kumawat G, Kumar S, Maheshwari, H. S, Ratnaparkhe, M. B. Crop improvement against *Colletotrichum truncatum* using molecular breeding approaches. In *QTL Mapping in Crop Improvement*. Academic Press. 2023;45-56.
5. Jones RA, Sharman M, Trębicki P, Maina S, Congdon BS. Virus diseases of cereal and oilseed crops in Australia: Current position and future challenges. *Viruses*. 2021;13(10):2051.
6. Nataraj V, Maranna S, Kumawat G, Gupta S, Rajput LS, Kumar S, Bhatia VS. Genetic inheritance and identification of germplasm sources for anthracnose resistance in soybean [*Glycine max* (L.) Merr.]. *Genetic Resources and Crop Evolution*. 2020; 67:1449-1456.
7. Rajput LS, Kumar S, Nataraj V, Shivakumar M, Maheshwari HS, Ghodki BS. Evaluation of Novel Fungicide for the Management of Soybean Anthracnose Disease and Yield Loss Estimation. *Legume Research-An International Journal*, 2022;1:8.
8. Adrees H, Haider MS, Anjum T. Inducing systemic resistance in cotton plants against charcoal root rot pathogen using indigenous rhizospheric bacterial strains and chemical elicitors. *Crop Protection*. 2019;115:75–83.
9. Rani L, Thapa K, Kanojia N. An extensive review on the consequences of chemical pesticides on human health and environment. *J Clean Prod*. 2021;283:124657.
10. Sabate DC, Petroselli G, Erra-Balsells R. Beneficial effect of *Bacillus* sp. P12 on soil biological activities and pathogen control in common bean. *Biological Control*. 2020; 141:104131.
11. Moin S, Ali SA, Hasan KA. Managing the root rot disease of sunflower with endophytic fluorescent *Pseudomonas* associated with healthy plants. *Crop Protection*. 2020;130:105066.
12. Bernardo-Mazariegos E, Valdez-Salas B, Gonzalez-Mendoza D. Silver nanoparticles from *Justicia spicigera* and their antimicrobial potentialities in the biocontrol of foodborne bacteria and phytopathogenic fungi. *Revista Argentina de Microbiologia*. 2019;51:103-109.

13. Dalal J, Kulkarni N. Effect of endophytic treatments on plant growth performance and disease incidences in soybean (*Glycine max* (L.) Merrill) Cultivar JS-335 against challenge inoculation with *R. solani*. American Journal of Agricultural and Biological Sciences. 2015; 10(2):99.
14. Brunda KS, Jahagirdar S, Kambrekar DN. Antagonistic activity of bacterial endophytes against major soil borne pathogens of soybean. Journal of Entomology and Zoology Studies. 2018;6(6):43-46.
15. Chauhan P, Bhattacharya A, Giri VP, Singh SP, Gupta SC, Verma P, Dwivedi A, Rajput LS, Mishra, A. *Bacillus subtilis* suppresses the charcoal rot disease by inducing defence responses and physiological attributes in soybean. Archives of Microbiology. 2022;204(5):266.
16. Khan Z, Doty SL. Characterization of bacterial endophytes of sweet potato plants. Plant Soil. 2009;322:197-207.
17. Dennis C, Webster J. Antagonistic properties of species-groups of *Trichoderma*: I. Production of non-volatile antibiotics. British Mycological Society. 1971;57(1):25-IN3.
18. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature. 1947;154:850.
19. Rajput LS, Kumar S, Bhati H, Nair K, Akhtar J, Kumar P, Dubey SC. Diversity assessment of indigenous and exotic Diaporthe species associated with various crops using ISSR, URP and SRAP markers. Indian Phytopathology. 2021; 74:615-624.
20. Maatallah J, Berraho EB, Munoz S, Sanjuan J, Lluch C. Phenotypic and molecular characterization of chickpea rhizobia isolated from different areas of Morocco. Journal Applied of Microbiology. 2002; 93:531-540.
21. de Almeida Lopes KB, Carpentieri-Pipolo V, Oro TH, Stefani Pagliosa E, Degrassi G. Culturable endophytic bacterial communities associated with field-grown soybean. Journal of applied microbiology. 2016;120(3):740-755.
22. Egamberdiyeva D, Hoflich G. Influence of growth-promoting bacteria on the growth of wheat in different soils and temperatures. Soil Biology and Biochemistry. 2003;35:973-978.
23. Sharma M, Mallubhotl S. Diversity, antimicrobial activity, and antibiotic susceptibility pattern of endophytic bacteria sourced from *Cordia dichotoma* L. Frontiers in microbiology. 2022;13:879386.
24. Hernández-Pacheco CE, del Carmen Orozco-Mosqueda M, Flores A, Valencia-Cantero E, Santoyo G. Tissue-specific diversity of bacterial endophytes in Mexican husk tomato plants (*Physalis ixocarpa* Brot. ex Horm.), and screening for their multiple plant growth-promoting activities. Current Research in Microbial Sciences. 2021;2:100028.
25. Singh SP, Gaur R. Evaluation of antagonistic and plant growth promoting activities of chitinolytic endophytic actinomycetes associated with medicinal plants against *Sclerotium rolfsii* in chickpea. Journal of Applied Microbiology. 2016; 121:506-518.
26. Feng X, Xu R, Zhao N, Wang D, Cun M, Yang B. Isolation, Identification, and Characterization of Endophytic *Bacillus* from Walnut (*Juglans sigillata*) Root and Its Biocontrol Effects on Walnut Anthracnose. Agriculture. 2022;12(12).
27. Abraham A, Philip S, Kuruvilla Jacob C, & Jayachandran K. Novel bacterial endophytes from *Hevea brasiliensis* as biocontrol agent against Phytophthora leaf fall disease. Biological Control. 2013;58: 675-684.
28. Soumyamol VB, Nejumunnisa PN, Roy CB. Biocontrol adeptness of bacterial endophytes antagonistic to *Colletotrichum* spp. causing Colletotrichum leaf disease in rubber (*Hevea brasiliensis*) and harnessing its plant growth-promoting traits. South African Journal of Botany. 2023;161:151-160.
29. Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different verticillium host plants. Applied and Environmental Microbiology. 2002;68: 3328-3338.
30. Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nature reviews. Microbiology. 2009;7:514-525.
31. Kumar A, Rithesh L, Kumar V, Raghuvanshi N, Chaudhary K, Pandey AK. *Stenotrophomonas* in diversified cropping

- systems: friend or foe Z? *Frontiers in Microbiology*. 2023;14.
32. Ray S, Singh P, Singh J, Singh S, Sarma BK, Singh, HB. Killed fungal pathogen triggers antifungal metabolites in *Alcaligenes faecalis* for plant defense. *Physiological and Molecular Plant Pathology*. 2023;125:101996.
33. Badri Fariman A, Abbasiliasi S, Akmar Abdullah SN, Mohd Saud H, Wong MY. *Stenotrophomonas maltophilia* isolate UPMKH2 with the abilities to suppress rice blast disease and increase yield a promising biocontrol agent. *Physiological and Molecular Plant Pathology*. 2022;121:101872.

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