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Isolation and characterization of bacterial endophytes from the *A. indica* for evaluation of their antimicrobial activity

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ABSTRACT

Despite of main economical crop, Capsicum annuum (Chilli) crop is challenged by various biotic and abiotic stresses. Among the biotic stress, Southern blight disease caused by Sclerotium rolfsii, and bacterial leaf spot caused by Xanthomonas sp. are major yield limiting factors. Endophytes are the group of endosymbiotic microbes present inside the host plant, which can be isolated, characterized and tested for their diverse bioactivities. In the present study fresh roots, stem and leaves samples of Azadirachta indica (neem) plant were collected from different drought stress prone areas of Baramati tehsil (Pune, Maharashtra). Out of fifteen isolated endophytic bacteria five isolates S1, S2, S3, S4, and S6 exhibited prominent antifungal effect. Quantitative analysis revealed isolate S6 inhibits the growth of Sclerotium rolfsii by 62.5% in antifungal assays. S1, S2, S3 and S4 showed significant antibacterial activity against Xanthomonas sp. (20mm, 20mm, 18mm and 13mm respectively). Through MALDI-TOF MS analysis, out of six selected endophytic bacteria three identified as strains within Bacillus sp. (S1, S2 and S4) and one as Acinetobacter junii (S5).

Keywords: *Capsicum annuum,* Southern blight disease, bacterial leaf spot, *Sclerotium rolfsii, Xanthomonas* sp.*, Azadirachta indica,* endophytic bacteria

INTRODUCTION

Capsicum annuum L. is a ubiquitous spice in India, belonging to the genus Capsicum within the Solanaceae family, commonly referred to as "red pepper". Additionally, it is a rich sources of essential vitamins including A, C and E. Despite its culinary and nutritional value, the chilli crop faces considerable challenges from a variety of fungal, bacterial, and viral diseases (Muthukumar *et al.,* 2010). Among the various fungal diseases, Southern blight disease caused by *Sclerotium rolfsii,* a polyphagous soil-borne fungal pathogen is most potent. The characteristics feature of it include white, hyphal growth of fungi, causing wilting and drying of leaves, eventual collapse of mature plants, and observable cracks near the collar region. Root damage occurs with unhealthy roots covered (Paparu *et al.,* 2020). On other hand one of the major bacterial disease i.e. bacterial leaf spot of chilli, caused by *Xanthomonas axonopodis pv. Vesicatoria,* is a significant disease first identified in 1914 in South Africa. It's a major threat to chilli production worldwide, attacking all parts of the plant. Leaf infections lead to defoliation, reducing the weight of marketable fruits, and increasing sun scald risk. The main economic impact is a decrease in fruit weight and quality, with up to 52% weight loss in infected fruits. Available chemicals and antibiotics against these diseases have less efficacy and stability (Chapke *et al.,* 2020). The relationship between plants and microorganisms, particularly bacteria, is essential for immunity, development and defence responses. Endophytic bacteria and fungi colonize host tissues internally without causing harm or symptoms of disease, unlike endosymbionts or pathogens which reside within living plant cells (Nayak S. and Bharali A., 2025). These endophytes are widespread among plants, though they occur at lower densities than rhizospheric bacteria. Neem (*Azadirachta indica)*, is a revered tree primarily found across the Indian subcontinent, belonging to the Meliaceae family. From last many centuries, every part of the neem tree has been utilized in traditional medicine, making it commercially exploitable. Endophytes inhabiting neem trees hold promise for synthesizing valuable metabolites which contribute to many pharmacological activities observed in their host (Linh *et al.,* 2020). This study was aimed to sample collection, enrichment and isolation of

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potent endophytes from neem plant followed by evaluation of their antimicrobial activity against selected chilli pathogens mainly bacterial leaf spot and southern blight.

MATERIALS AND METHODS

Sample collection

Fresh samples of roots, stems, and leaves were collected from *A. indica* (neem) plants found in stress-prone areas around Baramati, Pune (Maharashtra, India). Standard sampling techniques were employed, ensuring the collection of representative specimens. The samples were immediately placed in sealed plastic bags to maintain their freshness and transported to the laboratory and stored at 4°C in refrigeration condition (Duhan *et al.,* 2020).

Surface sterilization and processing of samples

Surface sterilization of the samples was performed under controlled environment within the laminar airflow. The procedure involved sequential steps like rinsing the samples with sterile distilled water for one minute followed by immersion in a 2% sodium hypochlorite solution and washing with 70% ethanol for three and one minute respectively. The samples were finally, rinsed with sterile distilled water three times to remove any residual sterilizing agents (Ramalashmi *et al.,* 2018).

Enrichment and Isolation of endophytes

Small pieces of surface sterilized stem, root and leaf (0.5-1.0 cm) were cut, dried in a sterile manner, and plated on LB (Luria Bertani) agar media. The plates were then incubated at 37 °C for 48 hours to allow for the maximum recovery of bacterial colonies. To confirm the disinfection process, aliquots of the sterile water used in the final rinse were plated onto LB agar (Singh *et al.,* 2017).

Pathogen Isolation

Sclerotium rolfsii

In the present study, *Sclerotium rolfsii* was isolated from infected chilli roots collected from Lasurne, Pune (Maharashtra, India). The samples were surface sterilized by using the method mentioned by Paparu, *et al.,* 2020. The surfaced sterilized roots samples were then placed on PDA (Potato Dextrose Agar). The isolated fungus was then characterized by using microscopic and wet mount techniques (Nájera, *et al.,* 2018 and Watanabe, 2002).

Xanthomonas sp.

In the present study, *Xanthomonas* was isolated from infected chilli leaves collected from Lasurne, Pune (Maharashtra, India). The samples were surface sterilized by using same protocol mentioned in section 2.2 (Ramalashmi, *et al.,* 2018). Every bacterial wound was placed into a sterilized mortar and blended in 0.2 mL of sterilized water. Subsequently, the obtained mixture was streaked on nutrient agar medium and the corresponding bacterial colonies were then isolated and subculture on NA medium (Awad-Allah, *et al.,* 2021). The probable isolates of *Xanthomonas* were further streaked on YDC (Yeast Dextrose Calcium Carbonate) medium to confirm its cell morphology (Soliman, *et al.,* 2022) and other colony characters. Characterization of isolated bacterium was done on the basis of biochemical tests using Bergey's Manual of Determinative Bacteriology (Kamble, *et al.,* 2024).

Screening of isolated endophytes

The potent endophytes were screened by using following assays

Antifungal assay

The well diffusion method was used to test the antifungal activity of the endophytes; freshly prepared sterile PDA medium were inoculated with the 1×10^6spores/ml of isolated fungus *Sclerotium rolfsii* (Siddiqui, *et al.,* 2016). Three wells of a 7 mm diameter were punched in the medium using a sterile cork borer, and 20 µl suspension of the isolated endophytic bacteria, positive control (Fluconazole 20 µg/ml) and negative control (distilled water) were added into separate well. The plates were then incubated at 27°C for 48 hours. After the incubation, the results were observed and the diameter of the zone of inhibition was measured (Premjanu, *et al.,* 2016).

Antifungal dual culture assay

From agar well diffusion method five isolates were screened and further tested to evaluate antagonistic activity against *Sclerotium rolfsii*. Each bacterial isolate was introduced onto

the separate NA plate using a sterile nichrome wire in a straight line, 2.5 cm away from it a 1 cm² fungal plug of *Sclerotium rolfsii* was placed. Following inoculation, the plates were incubated at 28 °C for 72 hours (Luu, *et al.,* 2021). A plate containing the fungus without bacteria inoculation served as the negative control. The cultures were then placed in an incubator at 30°C for six days, following which the percentage inhibition of radial growth (PIRG) was determined using the formula:

$$\text{PIRG } (\%) = \frac{(R1 - R2)}{R1} x100$$

Where, R1 represents the radial growth of *Sclerotium rolfsii* in the control plate, and R2 represents the radial growth of *Sclerotium rolfsii* towards the antagonist endophytic bacteria.

The percentage inhibition of radial growth was classified into different categories based on the level of antifungal activity: Less than 30%: low antifungal activity, 30% to less than 50%: moderate antifungal activity, 50% to less than 70%: high antifungal activity and 70% or more: very high antifungal activity. Bacterial isolates exhibiting more than 30% inhibition in this dual culture assay were selected for further analysis (Toh, *et al.,* 2016).

Antibacterial assay

The antibacterial activity of the isolated endophytic bacteria against the pathogen *Xanthomonas* was assessed using the well diffusion method on a nutrient agar medium. 0.1 ml culture of *Xanthomonas* was eventually spread on sterile NA medium plates. Three wells of five mm diameter were punched using a sterile cork borer. Amoxicillin served as the positive control at 20µg/ml, while sterile distilled water acted as the negative control. 20 µl test culture of endophytic bacteria (OD_{600}=0.5) was inoculated in the third well. All the plates were then incubated at 37°C for 24 hours, and the resulting zone of inhibition was measured using a scale (Mamarasulov, *et al.,* 2020).

Characterization of the selected endophytic isolates

The selected endophytes were successfully characterized up to species level by using morphological, biochemical and molecular methods (Ambawade and Pathade, 2015). Colony morphology including size, shape, Gram staining, motility etc. tests were used for morphological characterization, while catalase, oxidase, sugar fermentation, sugar utilization etc. tests were performed for biochemical characterization. The Molecular characterization of the isolates was performed by using MALDI-TOF analysis (Bobade, *et al.,* 2021).

RESULT AND DISSCUSSION

Sample collection

Five distinct stress-prone areas near Baramati tehsil were selected for sample collection from *Azadirachta indica*. Stem, root and leaves samples of *A. indica* were carefully collected and stored in sealed plastic bags for analysis.

Table 1: Collected samples (root, stem and leaves) from five different locations

Sr. No.	Sampling site	Type	Longitude	Latitude
1	Barhanpur	i.Root ii.Stem iii.Leaves	74.539099^0	18.216263^0
2	Sawantwadi	i.Root ii.Stem iii.Leaves	74.564682^0	18.213845^0
3	Gojubavi	i.Root ii.Stem iii.Leaves	74.559107^0	18.231332^0
4	Katphal	i.Root ii.Stem iii.Leaves	74.6238^0	18.237565^0
5	Sawal	i.Root ii.Stem iii.Leaves	74.642867^0	18.190045^0

Enrichment and Isolation of endophytes

Direct placement of cut-surface-sterilized sample pieces onto LB agar facilitated the growth of endophytic bacteria, and the viable colony counts after 24 and 72 hours are showed in table 2. A total of 15 isolates were obtained from five samples, subsequently purified and subcultured on LB agar. Sterility testing confirmed the efficacy of surface sterilization, as sterile distilled water used in the final rinse showed no microbial growth on LB agar after 24 hours of incubation.

Table 2: Enumeration of CFU (Colony Forming Units) from surface-sterilized plant samples on LB agar

Sample No.	Type	24 Hrs	72 Hrs
I	1. Root	00	06
	2. Stem	25	32
	3. Leaves	07	15
	4. Control	00	00
II	1. Root	09	10
	2. Stem	19	27
	3. Leaves	32	36
	4. Control	00	00
III	1. Root	24	45
	2. Stem	05	29
	3. Leaves	10	15
	4. Control	00	00
IV	1. Root	00	00
	2. Stem	02	10
	3. Leaves	03	08
	4. Control	00	00
V	1. Root	04	09
	2. Stem	08	16
	3. Leaves	03	10
	4. Control	00	00

Similar to our study, Linh, *et al.,* (2020) isolated 7 endophytic bacteria from *A. indica*, though their focus was on antimicrobial efficacy. Singh, *et al.,* (2017) also reported 25 Gram-positive, rod-shaped endophytes from neem, closely aligning with our findings. Duhan, *et.al,* (2020) used direct plating of surface-sterilized *Tinospora cordifolia* samples on LB agar, isolating 38 endophytic bacteria, which were further purified on nutrient agar-demonstrating the effectiveness of this method for endophyte isolation and study.

Pathogen Isolation

Sclerotium rolfsii

For *Sclerotium rolfsii* isolation infected chilli root samples were collected from Lasurne, Pune, MS, India (Lat.18.033822^{o} and Long. 74.772334^{o}). Samples showed white fungal growth on root (figure 1). The isolated fungus identified on the basis of morphological characteristics and it was found to be *Sclerotium rolfsii.* The isolated fungus was further used in evaluation of the antiufngal activity for screening of endophytes.

Collection of infected root samples from chilli plant

Wet mount of isolated fungus

S. rolfsii on potato dextrose agar

Figure 1: Isolation and characterization of *S. rolfsii*

Najera, *et al.,* (2018) and Paparu, *et al.,* (2020) used similar surface sterilization techniques to isolate and characterize *Sclerotium rolfsii*, focusing on its role in food rot in *Cucurbita argyrosperma* and southern blight disease in common beans, respectively. Soliman, *et al.,* (2022), and Awad-Allah, *et al.,* (2021) employed similar isolation techniques, using surface sterilization of plant samples to successfully isolate *Xanthomonas* species from pepper, tomato, and sweet pepper plants.

***Xanthomonas* sp.**

For *Xanthomonas* isolation infected chilli leaf samples were collected from Lasurne, Pune, MS, India (Lat.18.033822^{o} and Long. 74.772334$^{o)}$. Samples showed yellow spot on leaves fig. 4. Colonies reminiscent of *Xanthomonas* appeared on nutrient agar medium within a span of 3-6 days at 27°C. These colonies exhibited a convex, mucoid, glistening appearance, forming circular shapes with smooth edges and displaying a faint yellow pigment as shown in figure 2.

A B C

Figure 2: A) Typical bacterial leaf spot symptoms on leaf of *Capsicum annuum* B) Surface sterilized leaves for enrichment C)

Primary screening of *Xanthomonas* sp. on NA medium

Bacteria isolated from spot lesions of leaves of *C. annuum* exhibited characteristic *Xanthomonas* morphology when cultured on YDC medium. The colonies of all isolates displayed consistent features: a pale yellowish coloration, convex shape, mucoid texture, glistening appearance, wet surface and circular form with smooth edges. Microscopic examination revealed rod-shaped bacterial cells devoid of endospore formation, displaying motility, and testing negative with Gram staining. The isolates were characterized up to genus level by using biochemical tests performed according to Bergey's Manual of Determinative Bacteriology (9th edition). The organisms showed positive catalase and potassium hydroxide (KOH) tests, while tested negative for the oxidase test. The results of other biochemical test were showed in table 8. These observations collectively suggest the presence of *Xanthomonas* bacteria in the samples obtained from symptomatic chilli plants.

Table 3: Biochemical characterization of the *Xanthomonas* sp.

Biochemical tests	Results	Biochemical tests	Results
Catalase	Positive	Glucose fermentation	Positive
Oxidase	Negative	Arabinose	Positive
Potassium hydroxide (KOH)	Positive	Mannose	Positive
Starch hydrolysis	Positive	Galactose	Positive
Nitrate reduction	Negative	Cellobiose	Positive
Lysine decarboxylase	Negative	Fructose	Positive

Screening of isolated endophytes

Antifungal assay

For evaluation of antifungal activity against *Sclerotium rolfsii*, fifteen bacterial isolates were subjected to screening; five isolates (S1, S2, S3, S4, and S6) that exhibited significant inhibitory effect on fungal growth were selected for further study. Isolates S1, S2, and S6 showed significant inhibition compared to the positive control (fungicide).

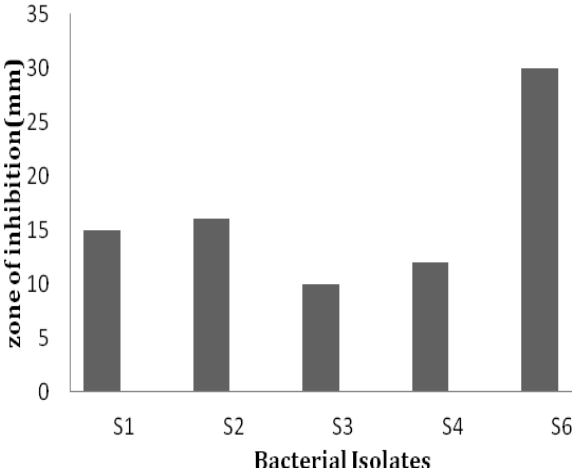

Figure 3: Zone of inhibition of endophytes against *S. rolfsii* by antifungal assay

For the screening of endophytic bacteria for antifungal activity against *Sclerotium rolfsii*, five isolates were initially selected based on their potential. These isolates were further evaluated using dual culture assays to assess their efficacy against *S. rolfsii*. Encouragingly, all five isolates demonstrated substantial inhibition of fungal growth, as showed in Table 4. Each isolate exhibited a percent inhibition of radial growth exceeding 30%, as depicted in Figure 4.Among the isolates tested, S6 displayed the most notable antagonistic effect against *S. rolfsii,* achieving a percentage inhibition of radial growth (PIRG) 62.5% followed by S4 and S2 also with PIRG values 48.75% and 47.5% respectively Table 4.

Antifungal dual culture assay

Figure 4: Dual culture assay of isolated endophytes against *S. rolfsii*

Table 4: Dual culture assay containing PIRG% value of isolates and inhibition length

Isolate used	Radial Growth (cm)	Inhibition length (cm)	PIRG % value
S1	5	1.1	37.5
S2	4.2	1.4	47.5
S3	5.3	1	33.75
S4	4.1	1.2	48.75
S6	3	2.5	62.5
Control	8	-	-

Archana, *et.al,* (2020) also used a dual culture assay to evaluate the antifungal effectiveness of seed bacterial endophytes against *Sclerotium rolfsii*, achieving inhibition rates of 25% to 60.1% (PIRG). Similar methodologies were employed by Luu, *et.al,* (2021), Al-Nadabi, *et.al,* (2021), and Toh, *et.al,* (2016), who assessed various endophytic bacteria for their antifungal activity while Edward *et al.* (2013) reported comparable mycelial growth inhibition against *Fusarium* species.

Antibacterial assay

The antibacterial assay was conducted to evaluate potential of isolated endophytes against *Xanthomonas* sp. From 15 isolates, five isolates (S1, S2, S3, S4 and S5) were selected for screening. Among these, S1, S2, S3 and S5 showed significant inhibitory activity when compared with positive control (Figure 5).

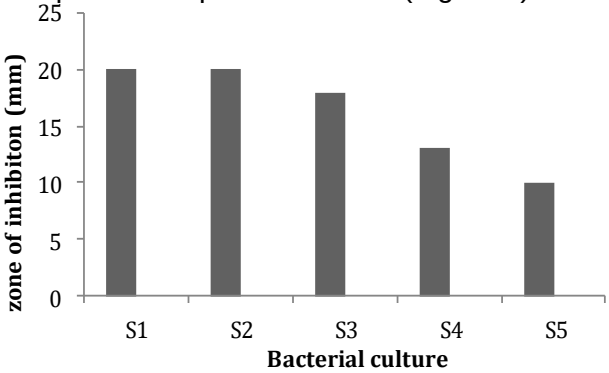

Figure 5: Zone of inhibition of endophytic bacteria against *Xanthomonas* sp.

Linh, *et al.,* (2020) assessed the antibacterial activity of endophytic bacteria from neem, found that only the KT2 strain inhibits *S. typhi* and *S. aureus* while Arunachalam, *et al.,* (2010) reported antibacterial activity in 5 out of 20 tested isolates showcasing the potential of endophytic bacteria in antimicrobial applications.

Table 5: Morphological characters of isolated endophytic bacteria

Isolate	Size	Shape	Colour	Margin	Elevation	Consistency	Opacity	Gram nature	Endospore forming
S1	3mm	Circular	Creamy white	Regular	Flat	Smooth	Opaque	Gram positive rods	Endospore forming
S2	2mm	Circular	Off white	Irregular	Flat	Sticky	Opaque	Gram positive rods	Endospore forming
S3	4mm	Circular	Faint pitch with edge	Regular	Convex	Smooth	Opaque	Gram positive rods	Non-Endospore forming
S4	3mm	Circular	Off white	Irregular	Flat	Smooth	Opaque	Gram positive rods	Endospore forming
S5	3mm	Circular	Off white	Regular	Convex	Smooth	Opaque	Gram negative rods	Endospore forming
S6	2mm	Circular	White	Regular	Convex	Smooth	Opaque	Gram positive rods	Endospore forming

Characterization of selected endophytic isolates

Morphological characteristics

The selected isolates were further identified by using the following morphological characteristic.

Biochemical test

All four isolates were further identified upto the genus level by using biochemical tests selected by Bergey's Manual of determinative bacteriology (Table 6). Based on the result obtained from biochemical test it was concluded that four isolates show similarities with *Acinetobacter* spp. and *Bacillus* spp.

Table 6: Biochemical characterization of the selected isolates

Test	Isolate S1	Isolate S2	Isolate S3	Isolate S4	Isolate S5	Isolate S6
Indole test	-	-	+	-	-	+
Methyl red	+	+	-	+	-	+
Voges Prousker	-	+	+	-	-	+
Catalase	+	-	+	+	+	+
Oxidase	+	+	-	+	+	-
Starch hydrolysis	-	-	+	-	+	-

(-) Negative, (+) Positive

Molecular analysis (MALDI-TOF MS)

The samples S1, S2, S3, S4, S5 and S6 were subjected to MALDI-TOF analysis at NCMR, Pune. The results obtained from MALDI-TOF analysis provide specific identification of the bacterial isolates based on their unique protein profiles. Each result includes the identification of the bacterial species, along with a corresponding score. This score represents the reliability of the identification, with higher scores indicating a closer match to the reference database. Out of six isolates four isolates were successfully identified to the species level using MALDI TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry). This method, renowned for its efficacy and reproducibility, was chosen for organism identification and two isolates were not reliable (suitable match was not found in Bruker's MALDI database) for identification which was not identified.

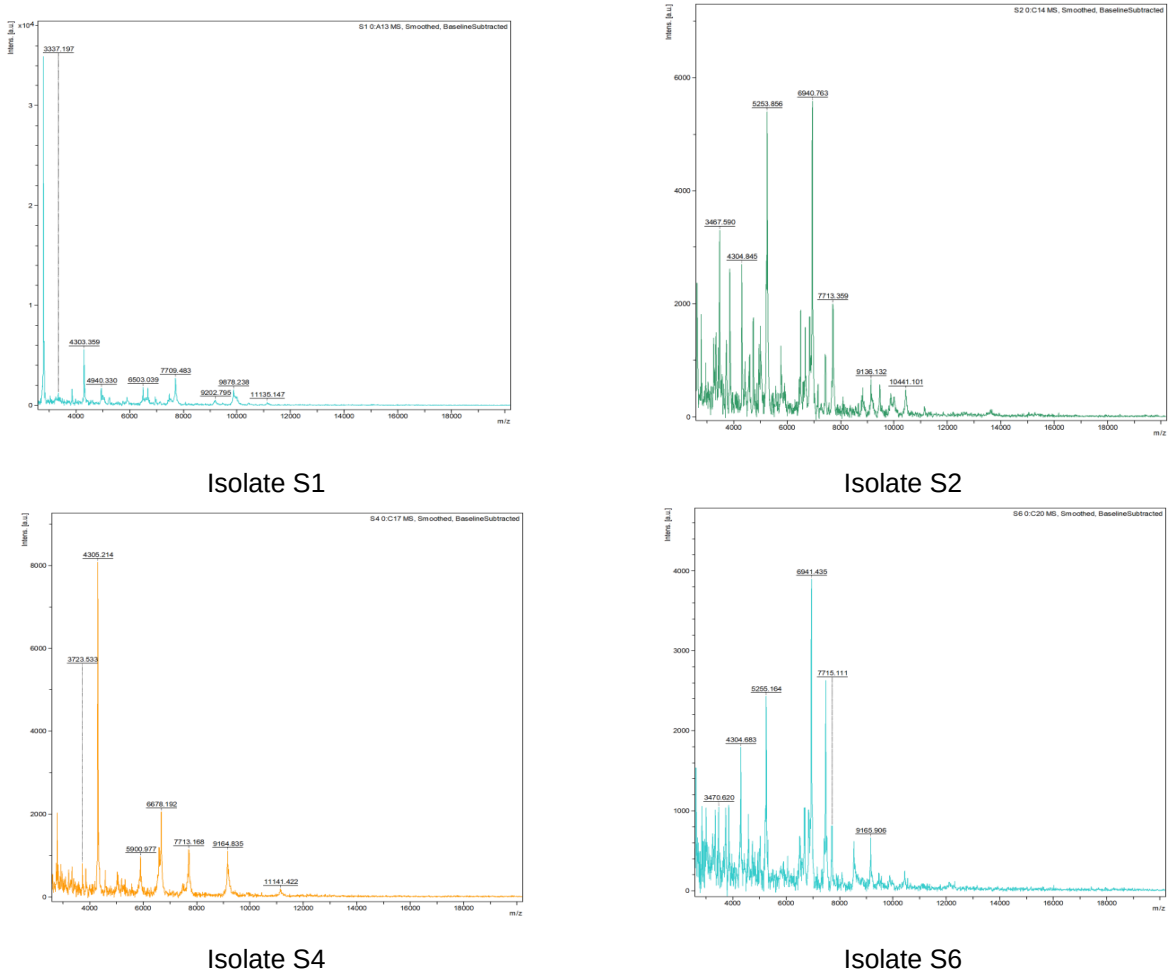

Figure 6: MALDI –TOF MS Spectra of isolated endophytes indicating protein profile (2 to 20 KDa.)

Table 7: Identification of isolated endophytes based on MALDI-TOF MS

Analyte (PRN)	Sample	Organism (best match)	Score	Organism (second match)	Score
M_APR_24_069	S1	*Bacillus subtilis* DSM 5552 DSM	1.997	*Bacillus subtilis* ssp *subtilis* DSM 10T DSM	1.907
M_APR_24_089	S2	*Bacillus subtilis* DSM 5611 DSM	1.910	*Bacillus subtilis* ssp *subtilis* DSM 10T DSM	1.855
M_APR_24_090	S3	Not reliable Identification	1.574	*Nil*	*Nil*
M_APR_24_070	S4	*Bacillus subtilis* DSM 5552 DSM	1.717	*Bacillus subtilis* ssp *subtilis* DSM 5660 DSM	1.713
M_APR_24_091	S5	*Acinetobacter junii* DSM 14968 HAM	2.021	*Acinetobacter junii* DSM 6964T HAM	1.921
M_APR_24_088	S6	Not reliable Identification	1.519	*Nil*	*Nil*

Anjum, *et al.,* (2015) optimized the isolation procedures, confirming endospore formation among many isolates, while Duhan, *et al.,* (2020) noted motility in the endophytic bacteria from *Tinospora cordifolia*. Recent studies by Martínez-Hidalgo, *et al.,* (2021), Sulistiyani, *et al.,* (2018), Wang, *et al.,* (2023), and Kiros, *et al.,* (2023) successfully utilized MALDI-TOF MS for identifying various endophytic bacteria, emphasizing its effectiveness in accurate bacterial classification and antibacterial assessments.

REFERENCES

Al-Nadabi, H.H., Al-Buraiki, N.S., Al-Nabhani, A.A., Maharach chikumbura, S.N., Velazhahan, R., and Al-Sadi, A.M. (2021) In vitro antifungal activity of endophytic bacteria isolated from date palm *(Phoenix doctylifera* L.) against fungal pathogens causing leaf spot of date palm. Egyptian *Journal of Biological Pest Control* **31**(65): 1-8.

Anjum, N. and Chandra, R. (2015) Endophytic bacteria: optimization of isolation procedure from various medicinal plants and their preliminary characterization. *Asian Journal of Pharmaceutical and Clinical Research* **8**(4): 233-238.

Archana, T., Rajendran, L., Manoranjitham, S. K., Santhana Krishnan, V.P., Paramasivan, M. and Karthikeyan, G. (2020) Culture-dependent analysis of seed bacterial endophyte, *Pseudomonas* spp. EGN 1 against the stem rot disease (*Sclerotium rolfsii* Sacc.) in groundnut. *Egyptian Journal of Biological Pest Control* **30**(119): 1-13

Arunachalam, C. and Gayathri, P. (2010) Studies on bioprospecting of endophytic bacteria from the medicinal plant of *Andrographi spaniculata* for their antimicrobial activity and antibiotic susceptibility pattern. International *Journal of Current Pharmaceutical Research* **2**(4): 63-68.

Awad-Allah, E. F., Shams, A. H. and Helaly, A. A. (2021) Suppression of bacterial leaf spot by green synthesized silica nanoparticles and antagonistic yeast improves growth, productivity and quality of sweet pepper. *Plants* **10**(8): 1689.

Bobade, S.S., Dhekane, S.P., Salunke, P.A., Mane, S.G., Dhawan, S.S., Marathe, R.J., Deshmukh, R.B. and Phatake, Y.B. (2021) Microbes Mediated Mitigation of Abiotic Stresses in Agriculture. *Indian Journal of Agricultural Research* **55**(6): 656-666

Chapke, S. M., Bharti, D. S., Sontakke, P. L., Patil, M. G. and Dhutraj, D. N. (2020) In vitro efficacy antibiotics against Bacterial leaf spot of chilli caused by *Xanthomonas axonopodispv. vesicatoria*. *International Jour. of Chemical Studies* **8**(6):1237-1239.

Duhan, P., Bansal, P. and Rani, S. (2020) Isolation, identification and characterization of endophytic bacteria from medicinal plant *Tinospora cordifolia*. *South African Journal of botany* **134**: 43-49.

Edward, E.J., King, W.S., Teck, S.L.C., Jiwan, M., Aziz, Z.F.A., Kundat, F.R., Ahmed, O.H. and Majid, N.A. (2013) Antagonistic activities of endophytic bacteria against *Fusarium wilt* of black pepper (*Piper nigrum*). *International Journal of Agricultural and Biological* **15**: 291-296.

Kamble, S. H., Surwase, S.N., Adagale V. G., Khune S. R., Sanika Arjun Bamankar, S. A., and Mahadik P. G. (2024) Evaluation of antimicrobial action of probiotics against *Xanthomonas axonopodis* pv. *punicae* causing bacterial blight of *Punica granatum* L. (Pomegranate). *Annals of Plant and Soil Research* **26**(4): 633-642.

Kiros, T., Ebu, S. M., Melaku, Y., Tesfa, T.and Dekebo, A. (2023) Isolation and identification of endophytic bacteria and associated compound from *Gloriosa superba* and their antibacterial activities. *Heliyon* **9**(11):1-10.

Linh, D. N., Van Thien, B., Ni, T. T. A., Duy, N. T., Van Minh, N. and Dong, T. C. (2020) Studies on antimicrobial activities of endophytic bacteria isolated from Neem tree (*Azadirachta indica*). Ho Chi Minh City Open University *Journal of Science-engineering and Technology* **10**(1): 72-80.

Luu, T. A., Phi, Q. T., Nguyen, T. T. H., Dinh, M. V., Pham, B. N. and Do, Q. T. (2021) Antagonistic activity of endophytic bacteria isolated from weed plant against stem end rot pathogen of pitaya in Vietnam. *Egyptian Journal of Biological Pest Control* **31**(14): 1-8.

Mamarasulov, B., Davranov, K. and Jabborova, D. (2020) Phytochemical, pharmacological and biological properties of *Ajuga turkestanica* (Rgl.) Brig (Lamiaceae). Annals of Phytomedicine **9**: 44–57.

Martínez-Hidalgo, P., Flores-Félix, J. D., Sánchez-Juanes, F., Rivas, R., Mateos, P.F., Santa Regina, I., and Velázquez, E. (2021) Identification of canola roots endophytic bacteria and analysis of their

potential as biofertilizers for canola crops with special emphasis on sporulating bacteria. *Agronomy* **11**(9): 1796-1802.

Muthukumar, A., Nakkeeran, S., Eswaran, A. and Sangeetha, G. (2010) In vitro efficacy of bacterial endophytes against the chilli damping-off pathogen *Pythium aphanidermatum*. Phytopathologia Mediterranea **49**(2):179-186.

Nájera, J.F.D., Castellanos, J.S., Hernández, M.V., Serna, S.A., Gómez, O.G.A., Verduzco, C.V. and Ramos, M. A. (2018) Diagnosis and integrated management of fruit rot in *Cucurbitaargyrosperma*, caused by *Sclerotium rolfsii. The Plant Pathology Journal* **34**(3): 171-180.

Nayak S. and Bharali A. (2025) Harnessing untapped potential of endophytes for disease management. *Annals of Plant and Soil Research* 27(1): 74-80.

Paparu, P., Acur, A., Kato, F., Acam, C., Nakibuule, J., Nkuboye, A.,.. and Mukankusi, C. (2020) Morphological and pathogenic characterization of *Sclerotium rolfsii*, the causal agent of southern blight disease on common bean in Uganda. *Plant disease* **104**(8): 2130-2137.

Premjanu, N., Jaynthy, C. and Diviya, S. (2016) Antifungal activity of endophytic fungi isolated from *Lannea coromandelica*–an insilico approach*. International Journal of Pharmaceutical Sciences* **8**(5): 207-210.

Ramalashmi, K., Prasanna, V. K., Magesh, K., Sanjana, R., Siril, J. S. and Ravibalan, K. (2018) A potential surface sterilization technique and culture media for the isolation of endophytic bacteria from *Acalypha indica* and its antibacterial activity*. Journal of Medicinal Plants Studies* **6**(1): 181-184.

Singh, A. K., Sharma, R. K., Sharma, V., Singh, T., Kumar, R. and Kumari, D. (2017) Isolation, morphological identification and in vitro antibacterial activity of endophytic bacteria isolated from *Azadirachta indica* (neem) leaves. *Veterinary world* **10**(5): 510–516.

Soliman, M. A. (2022) *Xanthomonas euvesicatoria* associated with bacterial spot on pepper fruits in Egypt. *Journal of Plant Protection and Pathology* **13**(1): 37-45.

Sulistiyani, T. R., and Lisdiyanti, P. (2018) Identification of endophytic bacteria from *Curcum azedoaria* based on protein profile using MALDI-TOF mass spectrometry. *Biotropia* **25**(2): 112-120.

Toh, S. C., Samuel, L. and Awang, A. S. (2016) Screening for antifungal-producing bacteria from *Piper nigrum* plant against *Phytophthora capsici*. *International Food Research Journal* **23**(6): 2616-2622.

Wang, X., Wu, G., Han, S., Yang, J., He, X., and Li, H. (2023) Differentiation and Identification of Endophytic Bacteria from Populus Based on Mass Fingerprints and Gene Sequences. *International Journal of Molecular Sciences* **24**(17): 13449-13453.

Watanabe, T. (2002) Pictorial atlas of soil and seed fungi. Morphologies of cultured fungi and key to species. 2nd ed. CRC Press, Washington DC, USA.