Annals of Phytomedicine 15(1): 815-823, 2026

DOI: http://dx.doi.org/10.54085/ap.2026.15.1.73

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Characterization of rice endophyte for its antifungal activity against rice false smut *Ustilaginoidea virens* (Cooke)

K. Kavitha*◆, S. Kaviyarasan*, S. Nazreen Hassan*, S. Suresh*, J. Selvi* and N. Rajinimala**

** ICAR-Krishi Vigyan Kendra, Thirupathisaram-629901, Kanyakumari, Tamil Nadu, India*

*** V.O. Chidambaranar Agricultural College and Research Institute, Killikulam-628252, Tamil Nadu, India*

Article Info

Article history
Received 11 March 2026
Revised 21 April 2026
Accepted 22 April 2026
Published Online 30 June 2026

Keywords
Ustilaginoidea virens
Oryza sativa L.
Endophytes
Bacillus safensis
False smut
Antimicrobial lipopeptides
Gas chromatography - Mass spectrometry

Abstract

The potential of endophytes as sustainable bioagent against rice false smut is explored in this work. Investigations provided evidence that, isolate *Bacillus safensis* RE-24 successfully inhibited rice false smut pathogen up to 59.05% under *in vitro*. *B. safensis* RE-24 produced extracellular hydrolytic enzymes and antimicrobial lipopeptides (AMPs) which have strong antibacterial and antifungal properties demonstrating its comprehensive antimicrobial potential. The *B. safensis* RE-24 isolate showed increased seedling growth and seedling vigour index. The results revealed that *B. safensis* can be further explored for its biological control activity.

1. Introduction

False smut is the reemerging disease in rice and incited by an ascomycetous fungus *Ustilaginoidea virens* (Cooke) (Fan *et al.,* 2016). *U. virens* produces hazardous mycotoxins, specifically ustiloxins (A, B, D, F, and E) and ustilaginoidins which has high impact in human health and causes yield loss up to 75%. Ustiloxins are water-soluble cyclopeptides that inhibit eukaryotic cell mitosis, making them both phytotoxic (inhibiting plant seed germination) and toxic to animals and humans whereas ustilaginoidins are fat-soluble pigments that are also toxic (Malathi and Gopinath, 2023). The chlamydospore survives in soil as well as seed throughout the season. Losses are both qualitative and quantitative. The chaffiness, reduction in test weight and spikelet sterility is due to infection. Major rice-growing states in India have reported severe cases of the disease since 2001 (Mandhare *et al.,* 2008).

The infection noticed during mid-October and severe during the dough and flowering stage. The pathogen infects stamen of the filament and colonizes the entire individual flowers called "False smut" (Nessa *et al.,* 2015). With the changing climate in India, false smut in rice has become a serious issue for certain rice varieties, leading to less yield (Tang *et al.,* 2013). Disease development is facilitated by temperatures between 25°C and 30°C, high relative humidity followed by intermediate rain (Hegde *et al.,* 2000).

Corresponding author: Dr. K. Kavitha
Associate Professor (Plant Pathology), ICAR- Krishi Vigyan Kendra, Thirupathisaram-629901, Kanyakumari, Tamil Nadu, India
E-mail: kavithak@tnau.ac.in
Tel.: +91-9003418457

False smut disease in rice can cause yield loss of 1 to 75%. Indiscriminate use of nitrogenous fertilizers and using high-yielding cultivars favors the disease development (Zhou *et al.,* 2014). It produces mycotoxins like ustiloxins and ustilaginoidins from the infected rice florets. *U. virens* preferentially attacks rice stamens (Zhou *et al.,* 2014). It colonizes in filaments and pollen tubes making infertile ovary and blocking nutrient transport (Wang *et al.,* 2016).

In recent years, host-associated microorganisms to manage plant diseases offers an environmentally friendly and safe approach. Endophytes are a strategic approach for mitigating biotic and abiotic stresses. It effectively produces bioactive chemical compounds. This created an opportunity to decrease pesticide usage and achieve sustainable agricultural production (Shahzad *et al.,* 2016). Endophytic microorganisms can effectively manage plant diseases by targeting both host-specific and non-host-specific pathogens through their mutual relationship, leading to sustainable development (Hardoim *et al.,* 2012). The endophytes can be substitute to fungicides to manage diseases. On perusal of the literature and evidence in commercialized products of bioagent, it is clear that several endophytes were isolated and evaluated against False smut. The endophytes are versatile multifaceted organisms having antagonistic potential against major diseases of the rice ecosystem. In this regard, the exploitation of endophytes will help to manage the disease in an environmentally safe approach and increase grain yield.

2. Materials and Methods

2.1 Isolation and characterization of pathogen

The young smut balls were gathered from infected rice fields and surface sterilized for one minute in 2% NaOCl and then rinsed in

70% ethanol for thirty seconds. After three rinses with sterile water, the surface-adhering disinfectant substance were dried in sterile filter paper. Using a sterile scalpel the smut balls was sliced and the tissue was kept in potato sucrose agar medium for four to five days at 28 ± 2°C. Hyphal tip was subcultured into a fresh medium as a pure culture and further stored at -20°C (Ladhalakshmi *et al.,* 2012).

The mycelia of completely grown plates were scraped using a sterilized needle and placed in the sterile pestle and motor and the mycelium was crushed using 1ml of 10% CTAB buffer for DNA isolation (Liu *et al.,* 2011). ITS-1F and ITS 4R primers were used to determine the molecular confirmity of the fungal pathogen. After purifying and sequencing the amplified PCR product (422 bp), the gene sequence was blasted in the NCBI database and compared.

2.2 Bacterial endophyte isolation

The rice seed samples gathered from different rice growing areas were pooled together for each traditional rice variety and used for isolation (Elbeltagy *et al.,* 2000). The seeds were disinfested in 2% NaOCl and then rinsed in 70% ethanol and sterile water. The surface-sterilized seed samples were crushed in a sterile pestle and mortar using phosphate buffer solution. After serially diluting samples, one milliliter was transferred to tryptic soy agar medium and one milliliter of water wash was used a blank control. Each morphologically unique colony was separated and kept at -20°C in a glycerol stock.

2.3 Screening of endophytes against *U. virens*

The biocontrol effectiveness of endophytes was investigated *in vitro* against *U. virens* by dual culture method (Dennis and Webster, 1971). The endophytes were selected based on fungal mycelial growth of *U. virens*. The percentage inhibition zone was computed and statistically analysed.

The per cent inhibition zone was calculated and statistically anlysied (Gomez and Gomez, 1984):

$$PIZ = \frac{C - T}{C} \times 100$$

PIZ- per cent inhibition zone, pathogen's mycelia growth (mm) in control plate is represented by C, while its growth in the presence of endophytes is represented by T.

2.4 Characterization of Endophytes

Bacterial endophytes that were 24 to 48 h old were put through to biochemical tests and their morphological features were examined using Gram staining (Aneja, 2007).

2.5 Screening of endophyte for hydrolytic enzymes and growth promoting activity

The production of hydrolytic enzymes, *viz.,* amylase (Alexander and Zuberer, 1991), protease (Jamali *et al.,* 2020), cellulase (Ariffin *et al.,* 2006), lipase (Hankin and Anagnostakis, 1975) by endophytic bacterial isolate were tested under *in vitro* condition. Similarly ammonia utilization (Jamali *et al.,* 2020), IAA Production (Siva *et al.,* 2024), siderophore production (Elgazzar, 2017) were assessed under *in vitro* condition by the endophyte.

2.6 Assessment of growth promoting activity of endophytes by paper towel method

The ability of endophyte to promote growth was assessed by paper towel method. Seeds were immersed in culture for 2 h and placed in germination paper. Vigor index was calculated on 7th day (Abdul–Baki and Anderson, 1973).

Vigor index = Germination (%) x Mean seedling length (Shoot + Root cm).

2.7 Molecular characterization of endophyte and detection of antimicrobial lipopeptides

Genomic DNA from effective endophyte was isolated by the lysozyme method (Xu *et al.,* 2020). The 16S rDNA region was amplified using universal primers 27F and 1492R (94^{o}C for 4 min, 35 cycles of 94^{o}C for 1 min, 55^{o}C for 1 min, 72^{o}C for 2 min and finally 72^{o}C for 10 min) in thermocycler (Bio Rad). After purifying and sequencing the amplified PCR product (1500 bp), the gene sequence was blasted in NCBI database and compared Genomic DNA of endophytic bacteria was used for the identification of genes involved in antibiotic biosynthesis using gene specific primers. The specific primers for iturin C, fengycin D and β1,3 glucanase (Baysal *et al.,* 2008), iturin D (Tsuge *et al.,* 2001), bacilysin, bacillomycin A, bacillomycin D and mycosubtilin (Chung *et al.,* 2008) were used to detect antimicrobial lipopeptide genes as per conditions described from the endophyte.

2.8 GC-MS based detection of secondary metabolites

The potential endophyte was used to extract the secondary crude antibiotic metabolite. According to Ajilogba *et al.* (2013), the endophyte was grown at 28 ± 2°C in LB broth for 78 h under constant shaking condition. The culture was mixed with ethyl acetate (HPLC) and shaken constantly throughout the whole night. A vacuum flask evaporator was used to dehydrate the solvent and methanol to dissipate. Derivatization was used to process the samples. Samples were dried fully for 3 h at 45°C in a concentrator. 50 µl of methoxamine hydrochloride was used to derivatize the samples and kept for 2 h at 37°C in shaking water bath. After adding 80µl of N-Methyl-N-(trimethylsilyl) trifluoroacetamide for 30 min at 37°C in a water bath the mixture was centrifuged for 3 min at 10000 rpm and the supernatant was used for GC-MS analysis at Department of Plant Biotechnology, TNAU Coimbatore.

2.9 Statistical analysis

Duncan's Multiple Range-Test (DMRT) was used to compare the treatment means (Gomez and Gomez, 1984).

3. Results

U. virens was isolated from infected grains (Figure 1) and microscopic observation showed septate mycelium with round yellow coloured double walled Chlamydospore (Figure 2) in MAGNUS MLX-B PLUS. ITS1 and ITS4 primers were used to validate the fungal pathogens molecularly. The amplified PCR products of 422 bp showed 98.53% identity with the *U. virens* isolate Uv5-US3-3E06 (OQ996290.1) in the NCBI database and submitted in gene bank *U. virens* (PQ432827) (Figure 3).

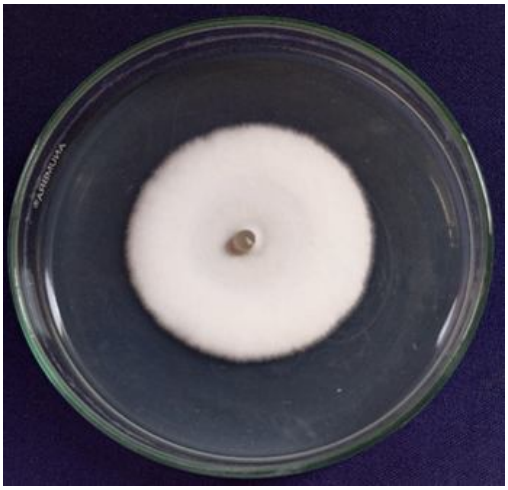


Figure 1: ***U. virens*** **isolated from infected smut grain balls in the potato sucrose agar media.**

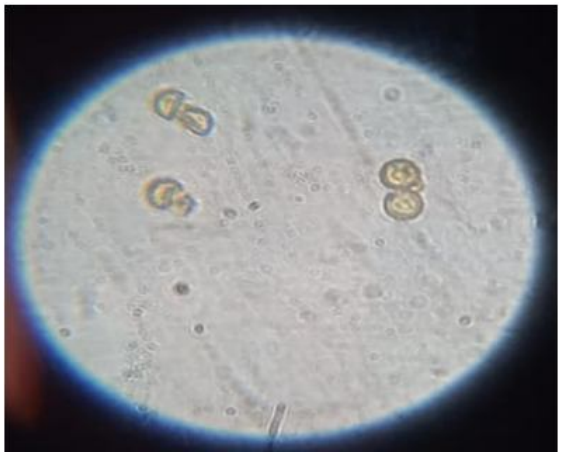


Figure 2: Microscopic observation of ***U. virens*** **with round yellow coloured double walled Chlamydospore.**

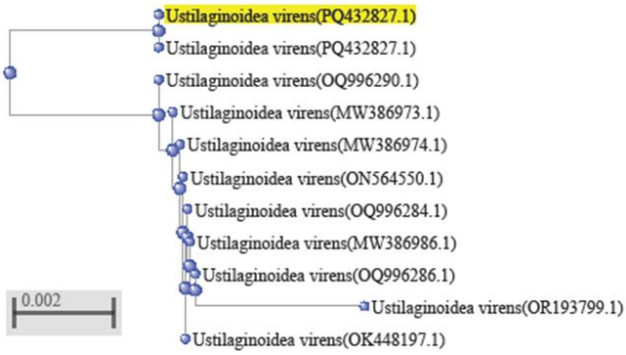


Figure 3: Phylogram of ***U. virens*** **generated based on ITS sequence data.**

Rice seed samples (9 Nos.) were gathered from different regions of Tamil Nadu, *viz*., Bhavanisagar of Erode District (11.23°N and 77.11°E) and Puthalam of Kanyakumari District (8.11° N and 77.46° E). From the seed samples, forty bacterial endophytes were isolated and renamed from RE-1 to RE-40 (Table 1).

Table 1: Endophytes from traditional rice

Traditional rice varieties	No. of endophytes isolated	Names of rice seed endophytes microflora (RE)
Oryza sativa L. (Karupukavuni)	8	1-8
Oryza sativa L. (Keerai samba)	4	9-12
Oryza sativa L. (Vellai milagu samba)	1	13
Oryza sativa L. (Kottara samba)	4	14-17
Oryza sativa L. (Sivan samba)	6	18-24
Oryza sativa L. (Attur kichilisamba)	3	25-27
Oryza sativa L. (Kotchi samba)	6	28-33
Oryza sativa L. (Arupathankuruvai)	5	34-38
Oryza sativa L. (Thuyamalli)	3	38-40

Forty seed-borne bacterial endophytes were evaluated against *U. virens* under *in vitro* condition. Only three endophytes showed antifungal activity. The isolate RE-24 showed higher per cent inhibition of 59.05 % with fungal mycelial growth of 14.33 mm, followed by RE-26 with 35.24% inhibition and 22.67 mm of mycelial growth. The radial mycelial growth of 35 mm was recorded in control plate (Figure 4). Endophytes that expressed maximum inhibition (RE-24) was selected as an effective endophyte against *U. virens* for further studies (Table 2).

Table 2: Evaluation of endophytes under *in vitro*

Effective endophytes	Mycelial growth (mm)	Inhibition (%)
RE-12	24.00^{c}	31.42 ^{c}
RE-24	14.33^{a}	59.05^{a}
RE-26	22.67^{b}	35.24^{b}
Control	35.00^{d}	0
SCD	0.45142	1.28953
CD	0.83964	2.39853

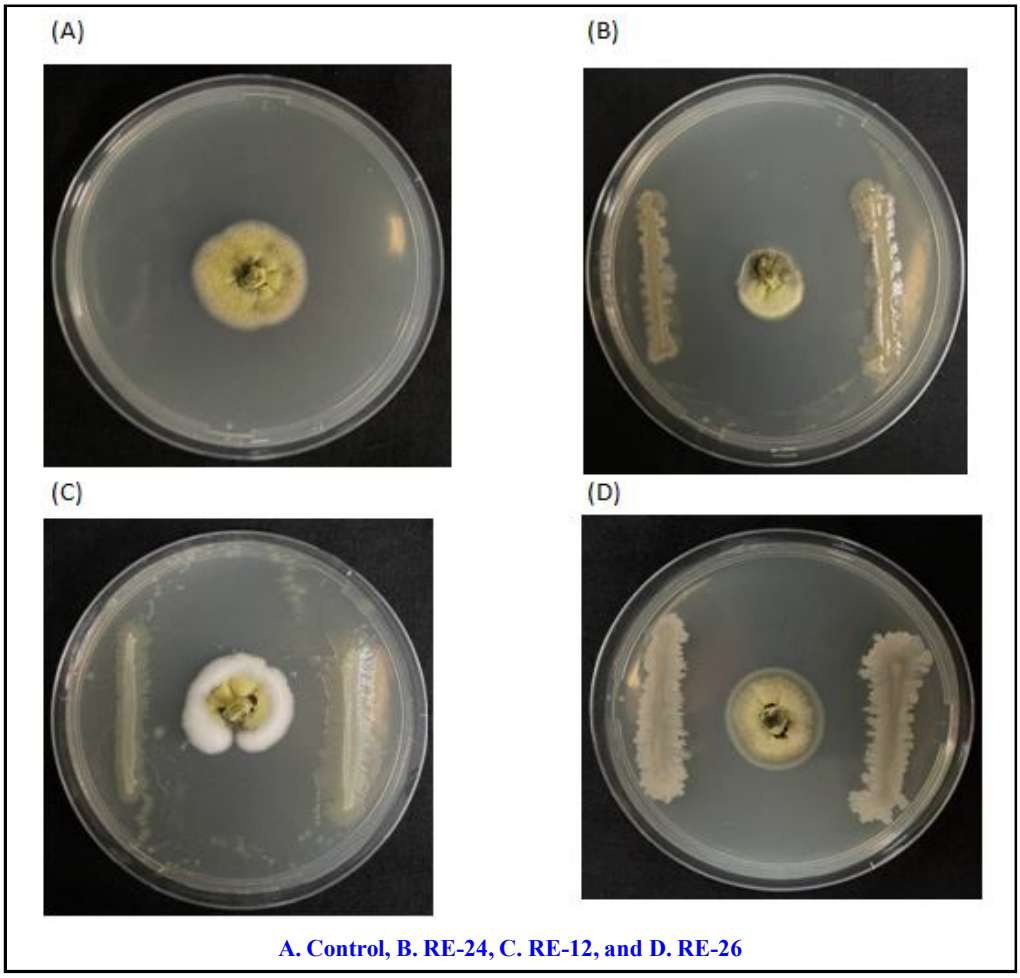
The results revealed that the performance of isolate RE-24 was significantly better when compared to other endophytic isolates. Consequently, a comprehensive study of the isolate RE-24 was performed. The isolate RE-24 showed series of dull creamy yellow colour colony growth, with positive reaction in gram staining. The isolate RE-24 exhibited positive reaction for the following tests, *viz*., urease test, citrate utilization test, glucose utilization test, protease production, ammonia utilization, IAA production test and siderophore production, whereas methyl red test, voges proskauer, cellulase production, lipase activity tests showed negative reaction (Table 3).

Table 3: Biochemical and plant growth promoting activity test

Treatments	*B. safensis* RE-24
Gram staining	Gram-positive
Urease test, citrate utilization test, glucose utilization test, protease production, ammonia utilization, IAA production test, siderophore production	Positive
Methyl red test, voges proskauer test, cellulase production test, amylase activity	Negative

The effective endophytic isolate RE-24 was molecularly identified by 16S rRNA. The amplified PCR products of 1055bp were sequenced and blasted and showed 97.30% identity with the *B. safensis* strain HBUAS78025 (PQ326613.1) and deposited to the NCBI database with the NCBI Accession number (PP783470) (Figure 5).

The roll towel method was carried out using germination paper for assessing the efficacy of effective endophytes (RE-24**)** for growth promotion. Isolate RE-24 was used to individually prime 25 seeds, and an untreated control was used as a check. The *B. safensis* RE-24 isolate treated seedlings increased the seedling growth, germination percentage and seedling vigour index (Table 4).



A. Control, B. RE-24, C. RE-12, and D. RE-26

Figure 4: *In vitro* screening of endophytes against *U. virens.*

Table 4: Effect of *B. safensis* RE-24 in plant growth promotion

Treatments	*B. safensis* RE-24	Control
Shoot length (cm)	3.98	3.45
Root length (cm)	11.72	10.85
Total height (cm)	15.07	14.30
Germination (%)	96	91
Vigour index	1446.53	1301.66

Antibiotic genes including bacillomycin A (344 bp), fengycin D (220 bp), iturin D (800 bp), and iturin C (506 bp) were amplified by *B. safensis* RE-24. The endophytes metabolites have high inhibitory activities against many plant pathogenic microorganisms. The experiment was conducted with one common control and endophytes RE-24. The endophyte isolate *B. safensis* RE-24 obtained 76 compounds, of which 93.42% were unique compound of endophyte RE-24, when compared with control and 6.57% compound showed similarity with the control. Out of the unique compound 28.16%

shows antibacterial, antifungal and antioxidant activity, remaining 71.83 has no report of such activity and hence it considering novel compound of that bacteria. 2,5-Piperazinedione, 3,6-bis(2-methyl-propyl) showed maximum area percentage of 12.57% with 32.422 retention time, followed by l-(+)-Ascorbic acid 2,6-dihexadecanoate 2.87% area and retention time 25.792 (Table 5). n-Heptylamine, N-acetyl-1-cyano has the lowest area percentage of 0.07 and a retention time of 29.032 (Figure 6).

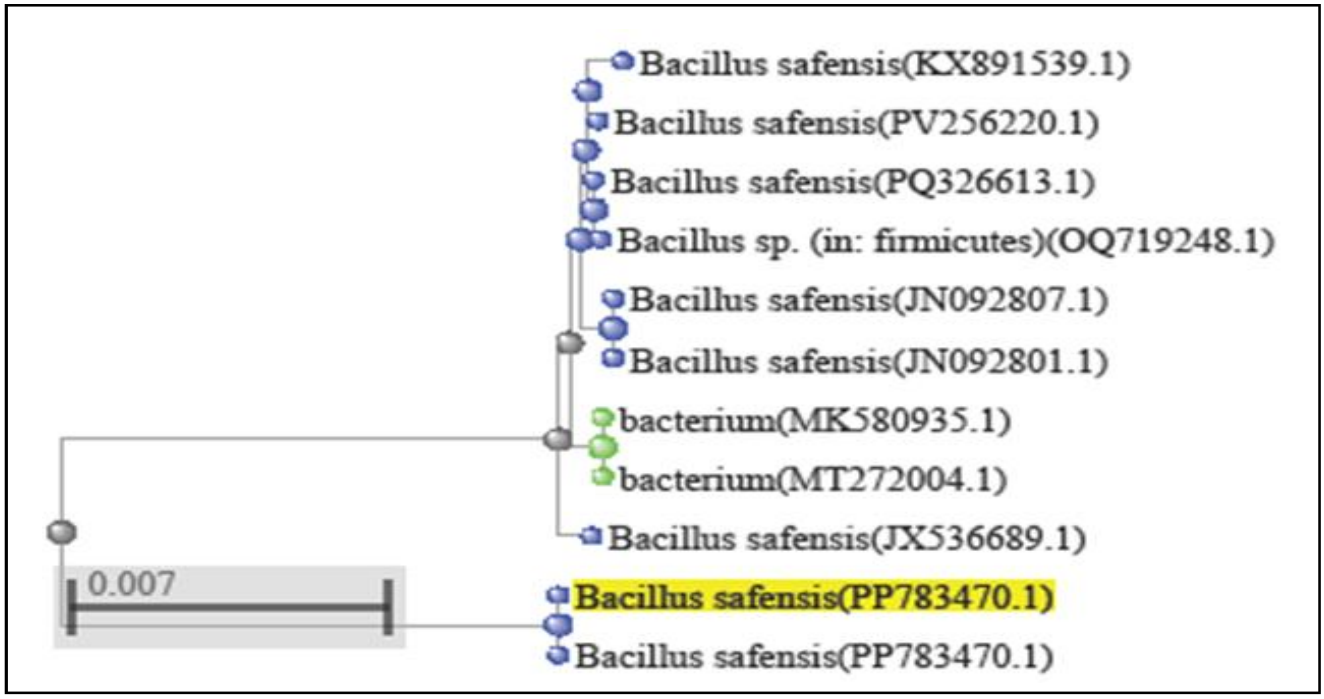


Figure 5: Phylogram of *B. safensis* generated based on 16S ribosomal RNA gene sequence data.

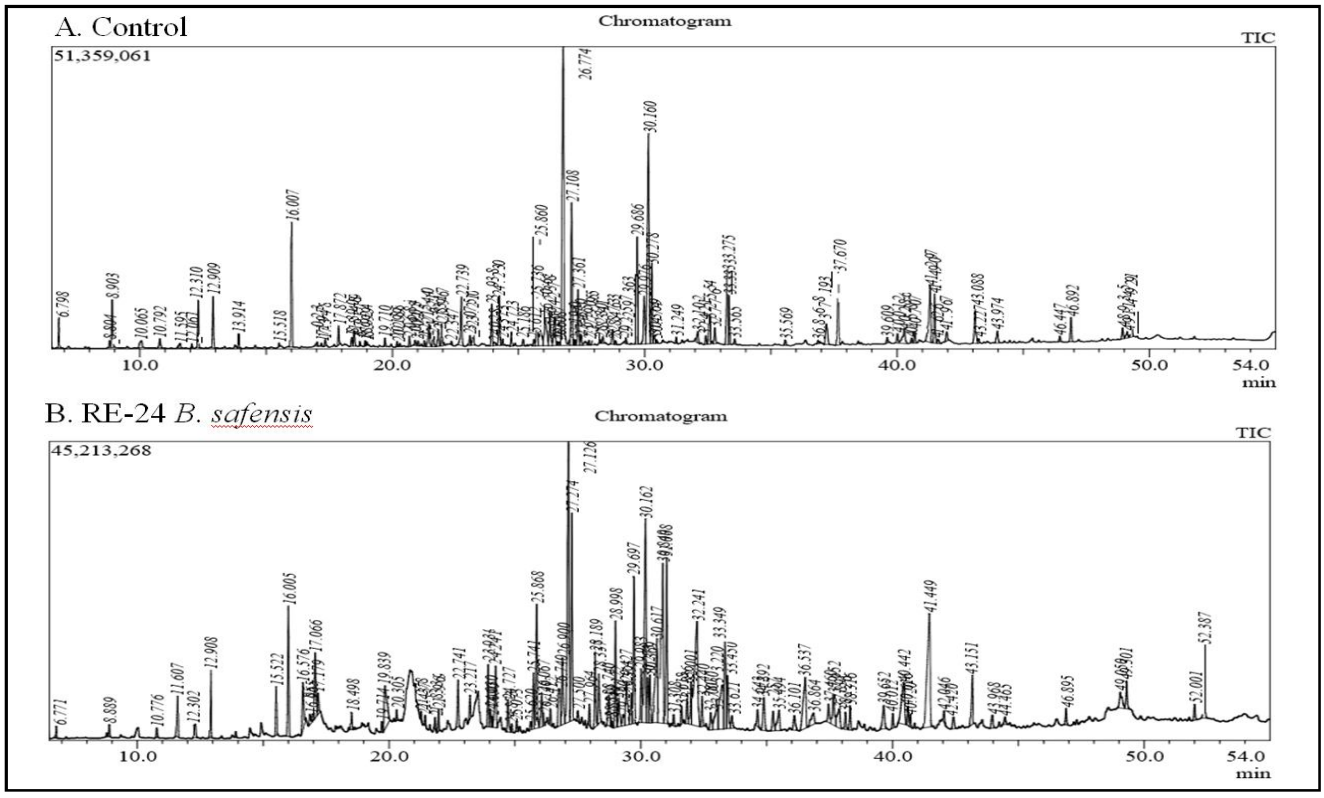
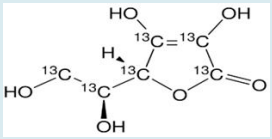
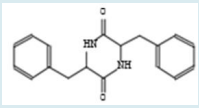


Figure 6: Chromatogram graph of GC-MS.

Table 5: GC-MS results for *B. safensis* RE-24 isolate

Compound	R/T	Area %	Type of compound	Activity	Structure	Reference
l-(+)-Ascorbic acid 2,6-dihex-adecanoate	25.792	2.87	Polyphenolic compounds	Antimicrobial activities		Khatri *et al.,* 2016
2,5-Piperazine-dione, 3,6-bis (2-methyl-propyl)-	32.422	12.57	Peptide	Antifungal activity		Srivastava *et al.,* 2017

4. Discussion

In the present scenario, false smut (*U. virens*), is the reemerging threat to rice cultivation (Mew *et al.,* 2002). Fungal pathogen false smut (*U. virens*) was confirmed using ITS 1 and ITS 4 primers and the amplified product size of more than 560 bp was sequenced, compared and submitted with Accession numbers *U. virens* (PQ432827).

The host-associated endophytes enhance the plant health. The bacterial endophytes enter the seed in different ways. The bacteria colonization takes place through nematodes, pathogenic chemotaxis, or the degradation of cell walls (Elbeltagy *et al.,* 2000). The bacterial root colonization enters into the vascular bundle, then spreads entire plant tissue, and finally enters into reproductive parts in the connection of vascular through funiculus and chalaza into endosperm *via* micropyle (Malfanova *et al.,* 2013).

The seed core endophytes were collected from different rice ecosystems of Erode, and Kanyakumari districts in Tamil Nadu. Forty endophytes were isolated from traditional rice varieties, *viz*., attur kichili samba, karupukavuni, sivan samba, vellai milagu samba, Thuyamalli, kotchi samba, keerai samba, kottara Samba, and arupathankuruvai. Seed endophytes from rice endosphere were explored for shaping resistance against plant pathogens (Walitang *et al.,* 2017; Matsumoto *et al.,* 2021).

The endophyte *B. safensis* RE-24 isolate showed high antifungal action by inhibiting the mycelial growth false smut (59.04%) under *in vitro* screening. Endophytic *Bacillus* spp. and *Pseudomonas* spp. showed high antifungal for *P. oryzae* in rice (Do, 2022) and *B. safensis* showed high inhibition to *P. oryzae* (Rong *et al.,* 2020). Similarly *Bacillus* spp. expressed high antifungal activity for rice false smut disease (Pandey *et al.,* 2024).

The morphology of RE-24 showed creamy yellow color morphology in the gram staining experiment. The biochemical tests were performed to distinguish the various categories of bacteria. Urease converts urea into ammonia and carbon dioxide and ammonical form of nitrogen is absorbed by plants (Bahmani *et al.,* 2021). Endophytes ferment glucose and transform the final product of glycolysis into gaseous byproducts such as formic acids, releasing hydrogen and carbon dioxide gas. Urease test was performed to study the ability of the bacteria to break down the urea in another form of nitrogen for easy uptake by the plants.

The isolates RE-24 exhibited positive reaction for urease test, citrate utilization test, glucose utilization test, protease production, ammonia utilization, IAA production test and siderophore production, whereas methyl red test, voges proskauer, cellulase production, amylase activity tests showed negative reaction. Similar reaction for biochemical tests were described (Kumar *et al.,* 2020 ; Manias *et al.,* 2020; Kobayashi and Palumbo, 2000).

Molecular identification of bacteria isolate RE-24 were done using 16s rRNA primers by the amplification size of 1055 base pairs. The results revealed isolate RE-24 matched with *B. safensis* and deposited with NCBI Accession number (PP783470) in NCBI database. The similar methods were used for characterization of entophytes (Kumar *et al.,* 2020; Rong *et al.,* 2020; Sirivella *et al.,* 2024). Cellulase, amylase, protease, and lipase are extracellular hydrolytic enzymes produced by the bacteria that support plant growth and prevent disease activity (Jamali *et al.,* 2020). The endophytic bacterial isolate RE-24 exhibited positive reaction for only protease activity and negative reaction for amylase and cellulase activity. Extracellular protease enzymes produced by *B. subtilis* increased resistance to *Ralstonia solanaceraum* (Prihatiningsih *et al.,* 2021). Enzymatic activity of *Bacillus* spp. was explored against dry root rot in glory lily and sheath blight of rice (Dhanabalan *et al.,* 2024; Jamali *et al.,* 2020). Similarly protease activity of *B. licheniformis* was studied (Sonune and Garode, 2018). *Bacillus* spp. showed cellulase, protease activity against *Fusarium oxysporum* f. sp. *lycopersici* (Ayantola and Fagbohun, 2021), hydrolytic action of *B. lichiniformis* and *Bacillus* spp. showed inhibitory activity against disease in fruit trees and forest trees (Ajuna *et al.,* 2023). Production of extracellular enzyme by microbes have wide potential role in the field of agriculture and other food and beverage industries.

The effective endophytes *B. safensis* RE-24 produced indole acetic acid, ammonia utilization, and siderophore activity. Plant growth and development activities were greatly enhanced by the production of phytohormones. Endophytes enhanced the growth-promoting activity in crops like tea (Kabir *et al.,* 2023), tomato (de O. Nunes *et al.,* 2023), groundnut (Archana *et al.,* 2020), sugarcane (Mendes *et al.,* 2007) and rice (Sirivella *et al.,* 2024). The biostimulant activity *viz*., Indole acetic acid, ammonia utilization activity, and siderophore production were depicted (Kumar *et al.,* 2020; Siva *et al.,* 2024). Endophytes improved the uptake of nutrients and enhanced colonization in plant tissue. Ammonia utilization by bacteria increases the chlorophyll content to boost up plant growth. *Pseudomonas aeruginosa* produces siderophores to prevent chili root rot and fruit rot. Siderophores are involved in both the biocontrol mechanism and promote plant development (Chandra *et al.,* 2010). *Bacillus* spp. reduced the infections of *R. bataticola, M. phaseolina* and *F. udam* in soybeans. (Govindasamy *et al.,* 2011). *Pseudomonas spp.* against sheath rot (Surya *et al.,* 2020). The endophyte *B. safensis* RE-24

increased the seedling growth. *B. licheniformis* and *B. subtillis* produced IAA and increased the growth of tomato (de O. Nunes *et al.,* 2023). Similar studies on growth promotion using *Bacillus* spp. have been reported in chickpea (Jana *et al.,* 2022) and cowpea (Siva *et al.,* 2024).

Antimicrobial peptides have antifungal and antibacterial activity against a variety of pathogens (Dhanabalan *et al.,* 2024; Kavitha *et al.,* 2025). *B. safensis* RE-24 amplified antibiotic genes such as iturin D (800 bp), iturin C (506 bp), fengycin D (220 bp), bacillomycin A (344 bp). PCR-based detection for the presence of lipopeptide genes in *Bacillus* spp. was reported (Ramyabharathi and Raguchander, 2014). *B. safensis* B21 produced iturin with high antifungal action (Rong *et al.,* 2020). Iturin and bacilysin produced by *Bacilius* spp. decreased the prevalence of soil-borne diseases of pepper and cucumber (Chung *et al.,* 2008). *Bacillus* spp. produced iturin and fengycin which showed antifungal action against rice blast (Lam *et al.,* 2021). *Bacilius* have the high antibiotic activity against *Xanthomonas* through the production of metabolite, *viz*., iturin, bacillomycin D and surfactin (Kumar *et al.,* 2020). Iturin and bacillomycin has strong metabolic activity against *R. solani* (Gond *et al.,* 2015). Similarly, iturin, surfactin, and b glucanase activity were reported in *Bacillus* spp. which have high antimicrobial activity against dry root rot in *Gloriosa superba* (Dhanabalan *et al.,* 2024). Mora *et al.* (2015) reported that *Bacillus* produced cyclic lipopeptide genes, *viz*., iturin, bacillomycin, fengycin, and surfactin shows anti-bacterial activity against plant pathogenic bacteria.

The secondary metabolite produced by endophytic bacteria had high antifungal and antibacterial activities. Reports on synthesis of strong antifungal compounds by *Bacillus* spp. was explored against *M. phaseolina* (Manikandan *et al.,* 2022), *Fusarium oxysporum* (Harish, 2022), *Sclerotium rolfsii* (Ramyabharathi and Raguchander, 2014), *Sclerotinia sclerotiorum* (Vinodkumar *et al.,* 2017), *Pythium aphanidermatum*, and *Rhizoctonia solani* (Vinayarani and Prakash, 2018). *Sarocladium oryzae* (Kaviyarasan *et al.,* 2025).

The secondary active metabolites secreted by *B. safensis* RE-24 isolate includes Benzeneacetic acid, Hydrocinnamic acid, Pyrrolidine, 1-(cyanoacetyl)-, 1-Dodecanol, 2,4-Di-tert-butylphenol, Succinylacetone-meto-TMS(3), 1-Heptadecene, Cyclo(L-prolyl-L-valine), l-(+)-Ascorbic acid 2,6-dihexadecanoate, 3,6-Diisopropylpiperazin-2,5-dione, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien, n-Hexadecanoic acid, Eicosane, n-Heptylamine, N-acetyl-1-cyano-, 13-Docosenamide, (Z)-, 9-Octadecenamide, (Z)-, 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-, Octadecanoic acid, Cyclo(prolyltyrosyl), Brevianamide F. Secondary active metabolites has high antifungal, antibacterial, antiviral, antimicrobial activities, antioxidant activity and nematicidal activity against plant pathogenic microbes (Srivastava *et al.,* 2017; Kaviyarasan *et al.,* 2024). *Bacillus* spp. produced high antifungal and antibacterial compounds against *R. solani* (Sirivella *et al.,* 2024), *Alternaria* sp. and *Helminthosporium* sp (Soliman *et al.,* 2022), *Sclerotinia sclerotiorum* and *Fusarium oxysporum* (Dos Santos *et al.,* 2023) and *P. oryzae* (Saleh, 2018).

B. safensis has the potential to be used for a variety of purposes, including disease management, industrial use, and improving plant nutrition. It proved that employing beneficial microbes to manipulate hosts can help reduce plant stress.

5. Conclusion

Research on rice endophytes *B. safensis* in particular shows how important they are in preventing false smut caused by *U. virens*. This study shows that endophytes not only have potent antifungal activity but also promote plant growth by secreting siderophores, producing growth-promoting hormones including indole acetic acid, and absorbing nutrients.

Acknowledgments

Funding support of Tamil Nadu Agricultural University, Coimbatore is highly acknowledged.

Conflict of interest

The authors declare no conflict of interest relevance to this article.

References

Abdul Baki, A. A. and Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria 1. Crop Science, **13**(6):630-633.

Ajilogba, C. F. ; Babalola, O. O. and Ahmad, F. (2013). Antagonistic effects of *Bacillus* species in biocontrol of tomato Fusarium wilt. Studies on Ethnomedicine, **7**(3):205-216

Ajuna, H. B. ; Lim, H. I. ; Moon, J. H. ; Won, S. J. ; Choub, V. ; Choi, S. I. and Ahn, Y. S. (2023). The prospect of hydrolytic enzymes from *Bacillus* species in the biological control of pests and diseases in forest and fruit tree production. International Journal of Molecular Sciences, **24**(23): 16889

Alexander, D. B. and Zuberer, D. A. (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biology and Fertility of soils, **12**:39-45.

Aneja, K. R. (2007). Experiments in microbiology, plant pathology and biotechnology. New Age International.

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**:1-13

Ariffin, H. ; Abdullah, N. ; Umi Kalsom, M. S. ; Shirai, Y. and Hassan, M. A. (2006). Production and characterization of cellulase by *Bacillus pumilus* EB3. Int. J. Eng. Technol, **3**(1):47-53

Ayantola, K. and Fagbohun E.J.A.J.o.R.i.B. (2021). *In vitro* enzymatic and antifungal activity of rhizobacteria against *Fusarium oxysporum* f. sp. *lycopersici*. Asian Journal of Research in Botany, **4**(1):182-91

Bahmani, K. ; Hasanzadeh, N. ; Harighi, B. and Marefat, A. (2021). Isolation and identification of endophytic bacteria from potato tissues and their effects as biological control agents against bacterial wilt. Physiological and Molecular Plant Pathology, **116**:101692

Baysal, Ö.; Çalýþkan, M. and Yeþilova, Ö. (2008). An inhibitory effect of a new *Bacillus subtilis* strain (EU07) against *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Physiological and Molecular Plant Pathology, **73**(1-3):25-32

Chandra, S.; Chatterje, S. and Acharya, K. (2010). Biological control of fruit rot and die back diseases of *Capsicum annuum* with *Pseudomonas aeruginosa* WS-1. Asian Australa's Journal of Plant Sciences and Biotechnology, **4**:38-42

Chung, S.; Kong, H.; Buyer, J. S.; Lakshman, D. K.; Lydon, J.; Kim, S. D. and Roberts, D. P. (2008). Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soilborne pathogens of cucumber and pepper. Applied Microbiology and Biotechnology, **80**:115-123

De O. Nunes, P. S.; De Medeiros, F. H.; De Oliveira, T. S.; de Almeida Zago, J. R. and Bettiol, W. (2023). *Bacillus subtilis* and *Bacillus licheniformis* promote tomato growth. Brazilian Journal of Microbiology, **54**(1):397-406

Dennis, C. and Webster, J. (1971). Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile antibiotics. Transactions of the British Mycological Society, **57**(1):25-39

Dhanabalan, S.; Muthusamy, K.; Iruthayasamy, J.; Kumaresan, P. V.; Ravikumar, C.; Kandasamy, R. and Periyannan, S. (2024). Unleashing *Bacillus* species as versatile antagonists: Harnessing the biocontrol potentials of the plant growth-promoting rhizobacteria to combat *Macrophomina phaseolina* infection in *Gloriosa superba*. Microbiological Research, **283**:127678

Do, Q. T. (2022). Antagonistic activities of endophytic bacteria isolated from rice roots against the fungus *Magnaporthe oryzae*, a causal of rice blast disease. Egyptian Journal of Biological Pest Control, **32**(1):69

Dos Santos, J. B.; de Oliveira Cruz, J.; Geraldo, L. C.; Dias, E. G.; Queiroz, P. R. M.; Monnerat, R. G. and Blum, L. E. B. (2023). Detection and evaluation of volatile and non-volatile antifungal compounds produced by *Bacillus* spp. strains. Microbiological Research, **275**:127465

Elbeltagy, A.; Nishioka, K.; Suzuki, H.; Sato, T.; Sato, Y. I.; Morisaki, H. and Minamisawa, K. (2000). Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. Soil Science and Plant Nutrition, **46**(3):617-629

Elgazzar, R. (2017). Screening of Siderophore Producers from Soil. Undergraduate Honors Theses. Paper, pp:379.

Fan, L. L.; Yong, M. L.; Li, D. Y.; Liu, Y. J.; Lai, C. H.; Chen, H. M. and Hu, D. W. (2016). Effect of temperature on the development of sclerotia in *Villosiclava virens*. Journal of Integrative Agriculture, **15**(11):2550-2555

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research. John Wiley and Sons.

Gond, S. K.; Bergen, M. S.; Torres, M. S. and White Jr, J. F. (2015). Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. Microbiological Research, **172**:79-87

Govindasamy, V.; Senthilkumar, M.; Magheshwaran, V.; Kumar, U.; Bose, P.; Sharma, V. and Annapurna, K. (2011). *Bacillus* and *Paenibacillus* spp.: Potential PGPR for sustainable agriculture. Plant growth and health promoting bacteria, Micromono., **18**:333-364

Hankin, L. and Anagnostakis, S. L. (1975). The use of solid media for detection of enzyme production by fungi. Mycologia, **67**(3):597-607

Hardoim, P. R.; Hardoim, C. C.; van Overbeek, L. S. and van Elsas, J. D. (2012). Dynamics of seed-borne rice endophytes on early plant growth stages. PloS one, **7**(2):e30438

Harish, S. (2022). Bio-prospecting of endospore-based formulation of *Bacillus* sp. BST18 possessing antimicrobial genes for the management of soil-borne diseases of tomato. Current Microbiology, **79**(12):380

Hegde, Y.; Anahosur, K. H. and Kulkarni, S. (2000). Influence of weather parameters on the incidence of false smut of rice. Advances in Agricultural Research in India, **14**:161-165

Jamali, H.; Sharma, A.; Roohi, N. and Srivastava, A. K. (2020). Biocontrol potential of *Bacillus subtilis* RH5 against sheath blight of rice caused by *Rhizoctonia solani*. Journal of Basic Microbiology, **60**(3):268-280.

Jana, S. K.; Islam, M. M. and Mandal, S. (2022). Endophytic microbiota of rice and their collective impact on host fitness. Current Microbiology, **79**(2):37

Kabir, M. H.; Unban, K.; Kodchasee, P.; Govindarajan, R. K.; Lumyong, S.; Suwannarach, N. and Khanongnuch, C. (2023). Endophytic bacteria isolated from tea leaves (*Camellia sinensis* var. *assamica*) enhanced plant-growth-promoting activity. Agriculture, **13**(3):533

Kavitha, K.; Kaviyarasan, S.; Indra, N.; Thilagavathi, R.; Rajinimala, N.; Preetha, G.; Suganya Kanna, S.; Nazreen Hassan, S. and Suresh, S. (2025). Induction of fungal disease resistance in rice mediated by bacterial endophytes. Plant Science Today, **12**(sp2):01-10

Kaviyarasan, S.; Kavitha, K.; Indra, N.; Meenakshisundram, P. and Thirukumaran, K. (2025). Isolation, characterization and metabolic profiling of seed endophyte *B. licheniformis* against *Sarocladium oryzae* in rice. Plant Science Today , **12**(sp1):01-11

Kaviyarasan, S.; Kavitha, K.; Indra, N.; Meenakshisundram, P. and Thirukumaran, K. (2024). Isolation and characterization of seed-borne endophytes for antifungal activity and plant growth promotion in rice, Pure Appl. Microbiol., **18**(4):2823-2836

Khatri, S. A. V. I. T. A.; Phougat, N. E. E. T. U.; Chaudhary, R. E. N. U.; Singh, B. H. A. R. A. T. and Chhillar, A. K. (2016). Chemical composition, antioxidant, antibacterial and cytotoxicity analysis of *Blumea lacera* (Burm. f.) DC. Int. J. Pharm. Pharm. Sci., **8**(8):313-19

Kobayashi, D. Y. and Palumbo, J. D. (2000). Bacterial endophytes and their effects on plants and uses in agriculture. In: Microbial Endophytes (pp:213-250). CRC Press

Kumar, V.; Jain, L.; Jain, S. K.; Chaturvedi, S. and Kaushal, P. (2020). Bacterial endophytes of rice (*Oryza sativa* L.) and their potential for plant growth promotion and antagonistic activities. South African Journal of Botany, **134**:50-63

Ladhalakshmi, D.; Laha, G. S.; Singh, R.; Karthikeyan, A.; Mangrauthia, S. K.; Sundaram, R. M. and Viraktamath, B. C. (2012). Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India. Phytoparasitica, **40**:171-176

Lam, V. B.; Meyer, T.; Arias, A. A.; Ongena, M.; Oni, F. E. and Höfte, M. (2021). *Bacillus* cyclic lipopeptides iturin and fengycin control rice blast caused by *Pyricularia oryzae* in potting and acid sulfate soils by direct antagonism and induced systemic resistance. Microorganisms, **9**(7):1441

Liu, K.H.; Yeh, Y.L. and Shen, W.C. (2011). Fast preparation of fungal DNA for PCR screening. J. Microbiol. Meth., **85**(2):170-172

Malathi, V. and Gopinath, M. P. (2023). A review on rice crop disease classification using computational approach. International Journal of Image and Graphics, **23**(03):2240007

Malfanova, N.; Lugtenberg, B. J. and Berg, G. (2013). Bacterial endophytes: who and where, and what are they doing there. Molecular Microbial Ecology of the Rhizosphere, **1**:391-403

Mandhare, V. K.; Gawade, S. B.; Game, B. C. and Padule, D. N. (2008). Prevalence and incidence of bunt and false smut in paddy (*Oryza sativa* L.) seeds in Maharashtra. Agricultural Science Digest, **28**(4):292-294

Manias, D.; Verma, A. and Soni, D. K. (2020). Isolation and characterization of endophytes: Biochemical and molecular approach. In: Microbial Endophytes (pp:1-14). Woodhead Publishing

Manikandan, A.; Jaivel, N.; Johnson, I.; Krishnamoorthy, R.; Senthilkumar, M.; Raghu, R. and Anandham, R. (2022). Suppression of Macrophomina root rot, Fusarium wilt and growth promotion of some pulses by antagonistic rhizobacteria. Physiological and Molecular Plant Pathology, **121**:101876

Matsumoto, H.; Fan, X.; Wang, Y.; Kusstatscher, P.; Duan, J.; Wu, S. and Wang, M. (2021). Bacterial seed endophyte shapes disease resistance in rice. Nature Plants, **7**(1):60-72.

Mendes, R.; Pizzirani-Kleiner, A. A.; Araujo, W. L. and Raaijmakers, J. M. (2007). Diversity of cultivated endophytic bacteria from sugarcane: Genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Applied and Environmental Microbiology, **73**(22):7259-7267

Mew, T. W. and Gonzales, P. (2002). A handbook of rice seedborne fungi. Int. Rice Res. Inst.

Mora, I.; Cabrefiga, J. and Montesinos, E. (2015). Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated *Bacillus* against phytopathogenic bacteria. PLoS One, **10**(5):e0127738

Nessa, B.; Salam, M. U.;Haque, A. M.; Biswas, J. K.; Latif, M. A.; Ali, M. A. and Galloway, J. (2015). Rice false smut disease at different flowering times. Bangladesh Rice Journal, **19**(2):28-35

Pandey, N.; Vaishnav, R.; Rajavat, A. S.; Singh, A. N.; Kumar, S.; Tripathi, R. M. and Shrivastava, N. (2024). Exploring the potential of *Bacillus* for crop productivity and sustainable solution for combating rice false smut disease. Frontiers in Microbiology, **15**:1405090

Prihatiningsih, N.; Djatmiko, H. A. and Lestari, P. (2021). Endophytic bacteria: An emerging tool for biological control bacterial leaf blight of paddy. In: IOP Conference Series: Earth and Environmental Science (Vol. 653, No. 1, pp:012080). IOP Publishing

Ramyabharathi, S. A. and Raguchander, T. (2014). Characterization of antifungal antibiotic synthesis genes from different strains of *Bacillus subtilis*. J. Pure Appl. Microbiol., **8**:2337-2344

Rong, S.; Xu, H.; Li, L.; Chen, R.; Gao, X., and Xu, Z. (2020). Antifungal activity of endophytic *Bacillus safensis* B21 and its potential application as a biopesticide to control rice blast. Pesticide Biochemistry and Physiology, **162**:69-77.

Saleh, M. M. (2018). Evaluation of endophytes isolated from rice leaves for their antifungal activities against *Pyricularia oryzae* causative blast disease. Egyptian Journal of Phytopathology, **46**(1):193-214

Shahzad, R.; Waqas, M.; Khan, A. L.; Asaf, S.; Khan, M. A.; Kang, S. M. and Lee, I. J. (2016). Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. Plant Physiology and Biochemistry, **106**:236-243

Sirivella, N.; Gopalakrishnan, C.; Kannan, R.; Pushpam, R.; Uma, D.; Raveendran, M. and Logeshwari, R. (2024). Analysis of bioactive secondary metabolites produced by endophytic *Bacillus amyloliquefaciens* against rice Sheath blight pathogen *Rhizoctonia solani*. Agricultural Science Digest-A Research Journal, **45**(1):131-137

Siva, M.; Sreeja, S. J.; Thara, S. S.; Heera, G. and Anith, K. N. (2024). Screening and evaluation of bacterial endophytes of cowpea (*Vigna unguiculata* (L.) Walp.) for plant growth promotion and biocontrol potential. Plant Science Today, **11**(2):44-57

Soliman, S. A.; Khaleil, M. M. and Metwally, R. A. (2022). Evaluation of the antifungal activity of *Bacillus amyloliquefaciens* and *B. velezensis* and characterization of the bioactive secondary metabolites produced against plant pathogenic fungi. Biology, **11**(10):1390

Sonune, N. and Garode, A. (2018). Isolation, characterization and identification of extracellular enzyme producer *Bacillus licheniformis* from municipal wastewater and evaluation of their biodegradability. Biotechnology Research and Innovation, **2**(1):37-44.

Srivastava, S.; Laisram, N.; Ram, H. and Singh, V. P. (2017). Bio-priming of tissue culture raised *Decalepis arayalpathra* (J. Joseph and V. Chandras.) Venter, KMA 05 clones with the phyllospheric bacterium, *Methylobacterium* sp. Society for Plant Research, **133**:41.

Surya, M.; Thiruvudainambi, S.; Ebenezar, E. G.; Vanniarajan, C.; Kumutha, K. and Vellaikumar, S. (2020). GC-MS Analysis of antimicrobial compounds produced by *Bacillus* spp. against rice sheath rot pathogen *Sarocladium oryzae*. J. Entomol. Zool. Stud., **8**:1417-1423

Tang, Y. X.; Jin, J.; Hu, D. W.;Yong, M. L.; Xu, Y. and He, L. P. (2013). Elucidation of the infection process of *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*) in rice spikelets. Plant Pathology, **62**(1):1-8

Tsuge, K.; Akiyama, T. and Shoda, M. (2001). Cloning, sequencing, and characterization of the iturin A operon. Journal of Bacteriology, **183**(21):6265-6273

Vinayarani, G. and Prakash, H. S. (2018). Fungal endophytes of turmeric (*Curcuma longa* L.) and their biocontrol potential against pathogens *Pythium aphanidermatum* and *Rhizoctonia solani*. World Journal of Microbiology and Biotechnology, **34**:1-17

Vinodkumar, S.; Nakkeeran, S.; Renukadevi, P. and Malathi, V. G. (2017). Biocontrol potentials of antimicrobial peptide producing *Bacillus* species: multifaceted antagonists for the management of stem rot of carnation caused by *Sclerotinia sclerotiorum*. Frontiers in Microbiology, **8**:446

Walitang, D. I.; Kim, K.; Madhaiyan, M.; Kim, Y. K.; Kang, Y. and Sa, T. (2017). Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of Rice. BMC Microbiology, **17:**1-13

Wang, W.; Zhai, Y.; Cao, L.; Tan, H. and Zhang, R. (2016). Endophytic bacterial and fungal microbiota in sprouts, roots and stems of rice (*Oryza sativa* L.). Microbiological Research, **188**:1-8

Xu, JX.; Li, Z.Y.; Lv, X.; Yan, H .; Zhou, G.Y. and Cao, L.X. (2020). Isolation and characterization of *Bacillus subtilis* strain 1-L-29, an endophytic bacteria from *Camellia oleifera* with antimicrobial activity and efficient plant-root colonization. PLoS ONE, **15**(4):e0232096

Zhou, Y. L.; Xie, X. W.; Zhang, F.; Wang, S. .; Liu, X. Z.; Zhu, L. H. and Li, Z. K. (2014). Detection of quantitative resistance loci associated with resistance to rice false smut (*Ustilaginoidea virens*) using introgression lines. Plant Pathology, **63**(2):365-372

Citation **K. Kavitha, S. Kaviyarasan, S. Nazreen Hassan, S. Suresh, J. Selvi and N. Rajinimala (2026). Characterization of rice endophyte for its antifungal activity against rice false smut *Ustilaginoidea virens* (Cooke). Ann. Phytomed., 15(1):815-823, http://dx.doi.org/10.54085/ap.2026.15.1.73.**