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Morpho-Molecular Characterization, *In-vitro* Screening of Biocontrol Agents from different Ecological Regions against Maize Stalk Rot Pathogen, *Fusarium verticillioides*

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Abstract

Biological control employing plant growth-promoting bacteria (PGPB), fungi and their inherent compounds has emerged as a promising strategy to mitigate ecological issues stemming from the over reliance on agricultural pesticides. This study explores the microbial diversity across diverse ecosystems in Karnataka, Andhra Pradesh and Tamil Nadu, encompassing forest, marine, marshy soils and river waters. Samples were collected from various locations, including Coorg, South Canara, Chikkamagalur, Mysore, Bapatla and Chennai districts, known for their unique environmental conditions. Morphological characterization revealed distinct traits among the isolated microbes, such as pigmentation, colony morphology and growth characteristics. Molecular characterization using ITS-1/ITS-4 and 16S rDNA primers identified a total of seven bacterial species and two fungal species, including *Bacillus subtilis*, *Burkholderia* spp., *Chryseo bacterium indologenes*, *Serratia marcescens*, *Stenotrophomonas maltophilia*. *In vitro* screening against *Fusarium verticillioides* demonstrated significant antagonistic activity, with several isolates showing high inhibition percentages. Phylogenetic analysis based on 16S rDNA and ITS sequences provided insights into the evolutionary relationships among these microbial strains. This comprehensive approach underscores the potential of these microbes in biocontrol and environmental applications, highlighting their role in ecological processes and sustainable agriculture.

Keywords : *In vitro* analysis, Antagonism, Maize stalk rot, Pathogen, BCA

Post-flowering stalk rot (PFSR) is a serious disease affecting maize production worldwide, primarily caused by the *Fusarium fujikuroi* species complex (FFSC), which includes over 60 Fusarium species. Key pathogens within FFSC responsible for PFSR include *Fusarium verticillioides*, *Fusarium subglutinans*, *Fusarium graminearum*, *Fusarium proliferatum* and *Fusarium oxysporum* (Khokhar *et al*., 2014a). These soil-dwelling fungi infect plants through roots and crowns, causing symptoms like drooping, wilting, leaf drying, empty cob development and increased angle between stalks and cobs. *Fusarium verticillioides* is a major PFSR pathogen, exhibiting significant variability in pathogenicity and genetics, across agroclimatic regions. PFSR is prevalent in many maize-growing states in India, especially in rainfed regions like Jammu and Kashmir, Punjab, Haryana, Rajasthan and others. During the *kharif* season, *Macrophomina phaseolina* is the primary PFSR pathogen, thriving in prolonged dry spells, while *F. verticillioides* predominates in irrigated areas. The disease affects 27-77 per cent of

maize plants, causing 22-64 per cent yield losses (Khokhar *et al*., 2014b). The variability in pathogenic potential and cultural characteristics of *Fusarium* spp. across different regions complicates control strategies.

Biological control using plant growth-promoting bacteria (PGPB) or their natural compounds has garnered significant attention as a promising solution to the ecological problems caused by excessive pesticide use in agriculture. These microorganisms, which naturally occur in the rhizosphere, form beneficial relationships with plants and represent a diverse group of advantageous bacteria (Rabhi *et al*., 2018). They enhance plant resistance against pathogens, reducing significant losses in plant productivity through various mechanisms. These mechanisms include root colonization and competition with other environmental microbes, production of antimicrobial peptides and induction of plant-mediated resistance responses (Köhl *et al*., 2019).

Burkholderia strains from the maize rhizosphere in France, were studied for their biocontrol potential and ability to induce systemic resistance against grey mould disease in grapevine. Strains demonstrated the capacity to inhibit spore germination and mycelium growth of *Botrytis cinerea* (Esmaeel *et al*., 2020). *Burkholderia cenocepacia*, effectively inhibits *Zymoseptoria tritici*, a significant pathogenic fungus responsible for *Septoria tritici* blotch in wheat and shows inhibitory activity against other phytopathogens (Song *et al*., 2024). *Burkholderia contaminans*, broad-spectrum antifungal activity, inhibited fungal pathogen growth in both dual culture and culture filtrate assays. Additionally, plant growth-promoting traits exhibited, including nitrogen fixation, phosphate solubilization, extracellular protease production, zinc solubilization and indole-3-acetic acid (IAA) biosynthesis (Heo *et al*., 2022). *Serratia marcescens* and *Bacillus* spp. demonstrated the highest potential for producing metabolites suitable as biocontrol agents in agriculture (Pereira *et al*., 2023). *Stenotrophomonas maltophilia* shows promise as both a biofertilizer and a biocontrol agent. These capabilities could sustainably enhance wheat production, ensuring food security in regions where wheat consumption is prevalent and the population is increasing (Sharma *et al*., 2024). Actinomycetes are found in diverse natural ecosystems, including rhizosphere soil, agricultural soil, marine and freshwater habitats, limestone, sponges, volcanic caves, deserts, insect guts, goat faeces and endophytic plants, according to *Purpureocillium lilacinum* is a biocontrol agent against various plant pathogens, such as root-knot nematode, *Meloidogyne incognita* and promotes plant growth in egg plants (Khan and Tanaka, 2023). Effective management is challenging due to the soil borne nature of the pathogen, making fungicide application costly and ecologically harmful. Research has shown regional variability in *Fusarium* spp. across Indian states, highlighting the need for comprehensive investigations to understand their virulence and develop management strategies. While biological control has been explored, breeding resistant germplasm is the ultimate solution to reduce crop loss due to PFSR. The present study aims to characterize the morphology of the strains comprehensively and to exploit potential biocontrol activity against *Fusarium verticillioides* causing PFSR.

Material and Methods

Sampling Site and Sample Collection

Samples were collected from various geographically distinct locations, each with unique environmental characteristics and undisturbed, human-uninterrupted areas contributing to a diverse array of microbial isolates. The collection sites included forest soils, marine soils, marshy soils and river water from different regions, specifically targeting areas in Karnataka, Andhra Pradesh and Tamil Nadu. Forest soil samples were predominantly collected from multiple sites in Coorg (Kolatodu Bygodu, Thalakaveri, Virajpete and Bhagamandala), South Canara (Sulya, Puttur and Charmady Ghat), Chikkamagalur (Charmady Ghat, Bababudangiri and Sitayyanagiri) and Mysore (Nagarahole forest). The soil samples were collected in aseptic plastic covers, river, marine water was collected in 50 ML Eppendorf tubes and sealed properly.

Isolation of Biocontrol Agents from the Soil

Soil samples were taken from different ecological locations using the purposive sampling method. One gram of the soil sample was suspended and diluted to 10^{-6}. Dilution levels 10^{-5} and 10^{-6} were placed into test tubes containing Potato dextrose media and Nutrient Agar (NA) media (Shankaranavar and Somasekhara, 2017). One ml of liquid was homogenized using a vortex, poured into a Petri dish and incubated for 3days.

Isolation of Biocontrol Agents from the Water

To isolate biocontrol agents from river water, first collect water samples from various locations within the river, ensuring a mix of different environmental conditions. Filter the water through a sterile mesh or filter paper to remove large debris and larger microorganisms. Then, pour the filtered water into sterile Petridishes or test tubes and incubate at an appropriate temperature (28±2°C) for several days to encourage the growth of microbial colonies (Saikat *et al*., 2024).

Purification and Maintenance of Biocontrol Agents

Purification and maintenance of biocontrol agents (BCAs) involve isolating and preserving the microorganisms to ensure their purity, stability and efficacy. After isolating potential BCAs from environmental samples, the culture is streaked onto Nutrient agar media to purify the organism. Once purified, the agent is maintained on suitable media such as agar slants for bacteria or potato dextrose agar (PDA) for fungi, with long-term storage at -80°C using glycerol stocks for bacterial strains or spore preservation for fungi. Regular subculturing (every 3-6 months) is required to retain the agent's vitality and periodic checks for contamination or loss of antagonistic properties are essential (Wang *et al*., 2022).

Morphological Characterization

Morphological characteristics such as pigmentation, colony colour, aerial mycelium, single colony shape, spread, serration and powdery growth. A total of 23 isolates were subjected to morphological characterisation and observations oncell shape, colony type, conidia, branching type, colony colour and pigmentation were made (Sudha and Narendrappa, 2015). The isolates were visualized on nutrient agar plates, observations were recorded after 5 days in triplicates.

Molecular Characterization

Isolation of DNA

Bacterial DNA isolated using the CTAB (Cetyltrimethy lammonium Bromide) method, begin by growing the bacterial culture overnight in an nutrient medium at 37 °C with shaking. Harvest the bacterial cells by centrifuging the culture at 4,000 rpm for 10 minutes and discard the supernatant. Resuspend the pellet in 1 mL of CTAB extraction buffer (containing 2% CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, pH 8.0). Add 10 µL of proteinase K (20 mg/mL) to the suspension and incubate at 60 °C for 30 minutes. After incubation, add an equal volume of chloroform: isoamyl alcohol (24:1) and gently mix for 10 minutes. Centrifuge at 12,000 rpm for 10 minutes to separate the phases. Transfer the upper aqueous phase to a clean tube and precipitate the DNA by adding 0.6 volumes of isopropanol and incubating at -20°C for 30 minutes. Centrifuge at 12,000 rpm for 10 minutes to pellet the DNA, then wash the pellet with 70 per cent ethanol and air-dry. Finally, dissolve the DNA in 50-100 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and store at -20°C (Wilson, 1987).

PCR Amplification of ITS and 16s rDNA Region

The ITS primers were ITS 1-5'- TCCGTAGG TGAACCTGCGG -3', ITS 4-5'- TCCTCCGC TTATTGATATGC -3' amplified for the fungal biocontrol agents (Larena *et al*., 1997). 16 s rDNA primers were 27F5'- AGAGTTTGATCCTGGC TCAG -3', 1492R-5'- TACGGYTACCTTGTTAC GACTT -3' were used to identify the bacterial strains (Woese and Fox, 1997). The PCR for ITS was initially set at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 3 minutes,

annealing at 55 °C for 1 minute and extension at 72 °C for 2 minutes, with a final extension at 72 °C for 10 minutes. The PCR for 16s rDNA was initially set at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 3 minutes, annealing at 57 °C for 1 minute and extension at 72°C for 2 minutes, with a final extension at 72°C for 10 minutes.

Sequencing and Data Analysis

The amplicon was electrophoresed on a 1.2 per cent agarose gel in 1 × TAE buffer, visualized using gel imaging and produced a band measuring approximately 1500 bp. All the amplicons were subjected to Sanger sequencing done at Europins India Pvt. Ltd. The phylogenetic tree was constructed using MEGA 11 (Tamura *et al*., 2021) using the neighbourhood joining method with 1000 boot strap values. *Fusarium verticiiloides* and *Bacillus subtilis* strains were used as outgroups in different phylogenetic trees and references were closely related species were selected.

Collection of *Fusarium verticillioides*

The most virulent isolate of stalk rot maize, *F. verticillioides* HSRF 18 was collected from the Pathogenomics Laboratory, Department of Plant Pathology, UAS, GKVK, Bengaluru for the screening of biocontrol agents.

***In-vitro* Screening of Biocontrol Agents against Pathogens**

In-vitro studies were carried out by using the dual plate technique. The experiment was conducted incompletely randomized design with three replications. The 3 days old biocontrol was streaked/ place don one side and the 7 days old Fusarium pathogen (5 mm disc) was placed onthe opposite side on PDA mediato evaluate the zone of inhibition (ZoI) (Khalil *et al*., 2021). Only pathogen was placed on PDA plate was considered as control and plates were incubated at 28±2 °C. Per cent inhibition of pathogen growth was calculated using the formula (Vincent, 1947) proposed.

Observations : Width of inhibition zone and mycelial growth of the test pathogen.

I = C-T/C X 100,

Where, C: Growth of mycelium in control (mm) and T : Growth of mycelium in treatment (mm)

Statistical Analysis

The data analysis was performed using R 4.1.0 (Anonymous, 2021) software. Tukey's test was used to determine the significant variances between the treatments and control, with $p<0.05$. All the experiments in this study were performed in triplicates.

Results and Discussion

Sample Site

A total of 21 Samples were collected from various geographically distinct locations, each with unique environmental characteristics and undisturbed, human uninterrupted areas contributing to a diverse array of microbial isolates. The collection sites included forest soils, marine soils, marshy soils and river water from different regions, specifically targeting areas in Karnataka, Andhra Pradesh and Tamil Nadu. River ecosystems are dynamic environments harbouring diverse microbial communities. These communities play critical roles in maintaining water quality, breaking down organic pollutants and supporting aquatic life. The geographic coordinates locations of the sampling points were depicted on map of India (Fig. 1, Table 1).

Isolation of Pure Novel Biocontrol Agents

The comprehensive sampling strategy covered a range of ecosystems, including forest soils, marine and marshy soils and river waters, across different geographical regions. Each environment presents unique physical and chemical conditions that shape the microbial communities. Collecting samples from such diverse environments provides valuable insights into the roles of these microbes in various ecological processes and their potential applications in fields such as biocontrol, agriculture and environmental

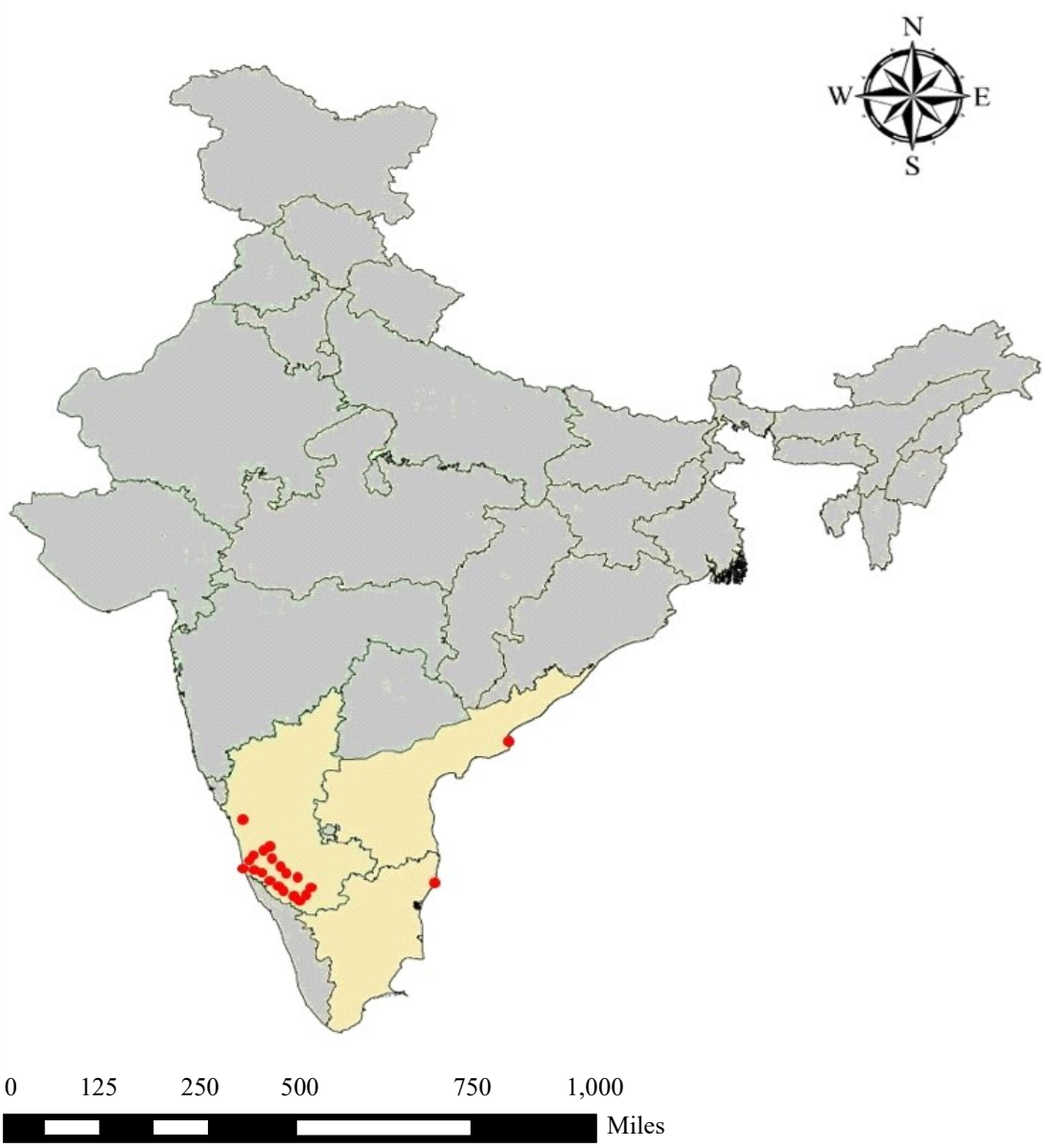


Fig. 1 : India map showing the coordinates of sampling site

sustainability. Fresh 500 grams of forest moist soil and marine soil were dug into 10 cm and the samples were collected in a sterile polythene cover. River water and Marine water was collected in Sterile falcon tubes. Later, the soil samples were subjected to randomisation and serial dilution for 10^{-5} to 10^{-6}. 1 ml of that water was poured into the NA medium. A total of 5 isolates were isolated from water and remaining 28 isolates were isolated from soil.

Morphological Characterisation

Various isolates were categorized by pigmentation, colony color, aerial mycelium presence, single colony shape, spread, serration and powdery growth. There are five different pigmentations and seven isolates were white pigmentation (APH1, APH10, APH25, APH26, APH28, APH39, APH46). three isolates were purple Pigmentation (APH7, APH14, APH27). two isolates were orange Pigmentation (APH12, APH35),

TABLE 1

GPS coordinates of samples for biocontrol agents collected from different ecological regions in Southern India

Location	Longitude	Latitude	Isolates	Isolation source
Nagarahole forest, Mysore	76° 5' 17.6964'' E	12° 15' 33.3972'' N	APH1	Forest soil
Kolatodu Bygodu, Coorg	75° 57' 27.2952'' E	12° 10' 4.8'' N	APH7	Forest soil
Thalakaveri, Coorg	75° 48' 53.2944'' E	12° 9' 43.9776'' N	APH10	Forest soil
Virajpete, Coorg	75° 48' 49.572'' E	12° 9' 38.8512'' N	APH12	Forest soil
Bhagamandala, Coorg	75° 39' 12.2436'' E	12° 21' 13.32'' N	APH14	Forest soil
Bhagamandala, Coorg	75° 36' 58.1904'' E	12° 21' 55.8576'' N	APH15	Forest soil
Sulya, South Canara	75° 37' 4.0944'' E	12° 27' 47.7936'' N	APH24, APH25, APH26	Forest soil
Puttur, South Canara	75° 15' 31.5756'' E	12° 40' 43.68'' N	APH27	Forest soil
Puttur, South Canara	75° 15' 31.5756'' E	12° 40' 43.68'' N	APH28	Forest soil
Panambur beach, South Canara	74° 49' 56.4384'' E	12° 49' 11.0712'' N	APH31	Marshy soil
Panambur beach, South Canara	74° 48' 24.9768'' E	12° 51' 54.9576'' N	APH34	Marine soil
Charmady ghat, South Canara	75° 26' 7.404'' E	13° 3' 38.5308'' N	APH36	Forest soil
Charmady ghat, Chikkamagalur	75° 29' 10.3272'' E	13° 5' 40.128'' N	APH38	River water
Charmady ghat, Chikkamagalur	75° 28' 56.7336'' E	13° 6' 43.9272'' N	APH39	Forest soil
Bababudangiri, Chikkamagalur	75° 45' 52.5384'' E	13° 25' 15.0024'' N	APH41	Forest soil
Sitayyanagiri, Chikkamagalur	75° 42' 34.7832'' E	13° 23' 10.5648'' N	APH42	Forest soil
Charmady ghat, Chikkamagalur	75° 28' 36.3'' E	13° 5' 42.6912'' N	APH43	River water
Panambur beach, South Canara	74° 47' 59.0208'' E	12° 54' 33.6528'' N	APH44	Marine Water
Baptla, Andhra Pradesh	80° 31' 3.1944'' E	15° 50' 28.3632'' N	APH45	Marine Water
Sirsi, North canara	74° 44' 3.264'' E	14° 38' 6.4932'' N	APH46	Forest soil
Chennai, Tamil Nadu	80° 17' 4.794'' E	13° 3' 17.5356'' N	APH26	Marine Water

elevenisolates were yellow pigmentation (APH16, APH15, APH24, APH31, APH36, APH38, APH41, APH42, APH43, APH44, APH45). Aerial mycelium is present in four isolates (APH1, APH12, APH25, APH35). In 19 isolates were aerial mycelium is absent (APH7, APH14, APH27, APH10, APH26, APH28, APH39, APH46, APH16, APH15, APH24, APH31, APH36, APH38, APH41, APH42, APH43, APH44, APH45). Single colony shape was round in most isolates and uneven in nine isolates (APH10, APH16, APH15, APH24, APH26, APH28, APH36, APH38, APH45). Serration was present in five isolates (APH7, APH10, APH26, APH27, APH28) and absent in remaining isolates. Powdery growth present in five isolates (APH1, APH12, APH28, APH35, APH46) and absent in remaining 18 isolates (APH7, APH14, APH15, APH16, APH24, APH25, APH26, APH27, APH31, APH36, APH38, APH41, APH42, APH43, APH44, APH45, APH39 and APH10). White pigmented isolates commonly show both aerial mycelium presence and round or uneven shapes. They are spread regularly and irregularly, with serration often absent, except in a few cases. Purple pigmented Isolates had round shapes and regular spread, with no aerial mycelium and minimal powdery growth. Orange pigmented isolates vary in colony colour from dull white to yellow-green, generally irregular spread and show aerial mycelium presence. Yellow pigmented

isolates display a range of characteristics, mostly without aerial mycelium, regular spread, round or uneven shapes and rarely have serration (Fig. 2, Table 2). The morphological presence of pigment colony size was then compared with the standard description of Bergey's Manual of Determinative Bacteriology (Bergey, 1994). The diversity of identified species indicates the robustness of the primers in detecting a wide range of microorganisms.

Molecular Characterization

Using universal primer pairs ITS-1/ITS-4 and 16s rDNA primers successfully amplified DNA bands of indigenous microbe isolates measuring ± 600 bp and ±1465 bp, respectively. The ITS-1/ITS-4 primer pair detected DNA in the internal region. Transcribed spacer (ITS), which in this region does not encode functional proteins and is in the ribosomal RNA

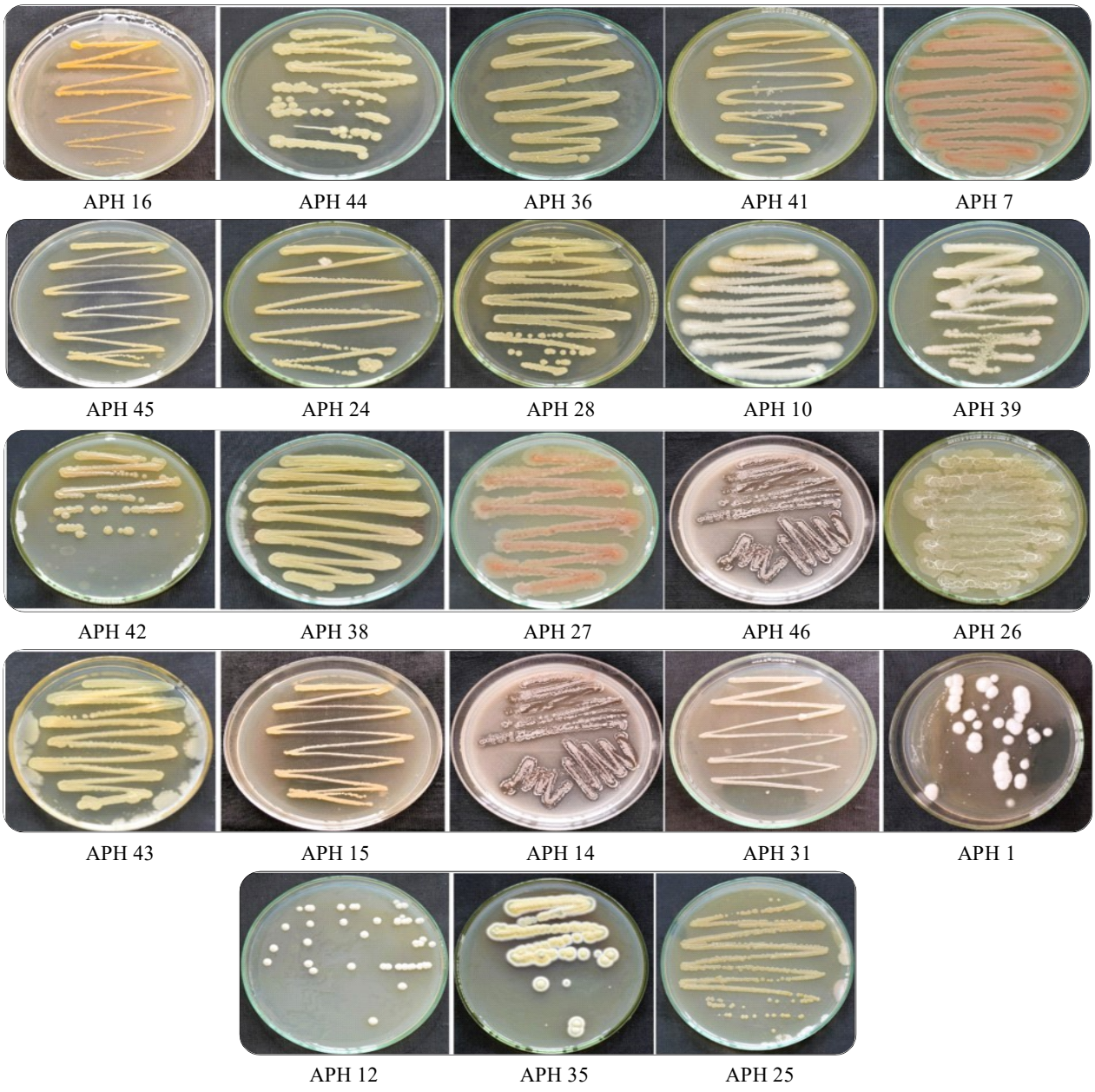


Fig. 2 : Morphological characters of all 23 purified isolates

TABLE 2

Morphological characters of all 23 isolates

Isolate	Pigmentation	Colony colour	Aerial mycelium	Single colony shape	Spread	Serration	Powdery growth
APH12	Orange	Dull white	Present	Round	Irregular	Absent	Present
APH35	Orange	Yellow-green	Present	Round	Irregular	Absent	Present
APH7	Purple	Purple	Absent	Round	Regular	Present	Absent
APH14	Purple	Purple	Absent	Round	Regular	Absent	Absent
APH27	Purple	Purple	Absent	Round	Regular	Present	Absent
APH1	White	White	Present	Round	Irregular	Absent	Present
APH10	White	White	Absent	Uneven	Regular	Present	Absent
APH26	white	White	Absent	Uneven	Regular	Present	Absent
APH28	White	White	Absent	Uneven	Regular	Present	Present
APH39	White	White	Absent	Round	Regular	Absent	Absent
APH46	white	White	Absent	Round	Regular	Absent	Present
APH25	White	White	Present	Round	Regular	Absent	Absent
APH16	Yellow	Yellow	Absent	Uneven	Regular	Absent	Absent
APH15	Yellow	yellow	Absent	Uneven	Regular	Absent	Absent
APH24	Yellow	Yellow	Absent	Uneven	Regular	Absent	Absent
APH31	Yellow	Yellow	Absent	Round	Irregular	Absent	Absent
APH36	Yellow	Yellow	Absent	Uneven	Regular	Absent	Absent
APH38	Yellow	Yellow	Absent	Uneven	Regular	Absent	Absent
APH41	Yellow	Yellow	Absent	Round	Regular	Absent	Absent
APH42	Yellow	Yellow	Absent	Round	Irregular	Absent	Absent
APH43	Yellow	Yellow	Absent	Round	Regular	Absent	Absent
APH44	Yellow	Yellow	Absent	Round	Irregular	Absent	Absent
APH45	Yellow	Yellow	Absent	Uneven	Regular	Absent	Absent

(rRNA) region. A total of 23 isolates were subjected to PCR amplification. Seven species from six different bacteria genera and two fungi genera were identified. *Bacillus subtilis* (APH26), *Burkholderia cenocepacia* (APH14, APH28 and APH44), *Burkholderia cepacia* (APH24, APH31, APH36 and APH42), *Burkholderia contaminans* (APH39 and APH45), *Chryseobacteriu mindologenes* (APH46), *Serratia marcescens* (APH7, APH10, APH16, APH27, APH38, APH41 and APH43), *Stenotrophomonas maltophilia* (APH15), *Fusarium oxysporum* (APH1, APH35 and APH 25) and *Purpureocillium lilacinum* (APH 12). The phylogenetic tree was constructed based on 16S rDNA and ITS sequences separately, using comparative 16S rRNA and ITS gene sequence analysis for bacterial and fungal identification at the genus and species levels (Fig. 3, Table 3).

Inferring phylogenetic relationships among fungi and prokaryotic organisms. Typically, strains with 16S rRNA and ITS-rDNA sequence similarities below 97 per cent are considered different species, while those above 97 per cent likely belong to the same species. The evolutionary history was inferred using the neighbour-joining method and the Tamura-nei model. The tree includes full 16S rDNA and ITS-rDNA gene sequences separately, illustrating relationships among strains and related taxa, with

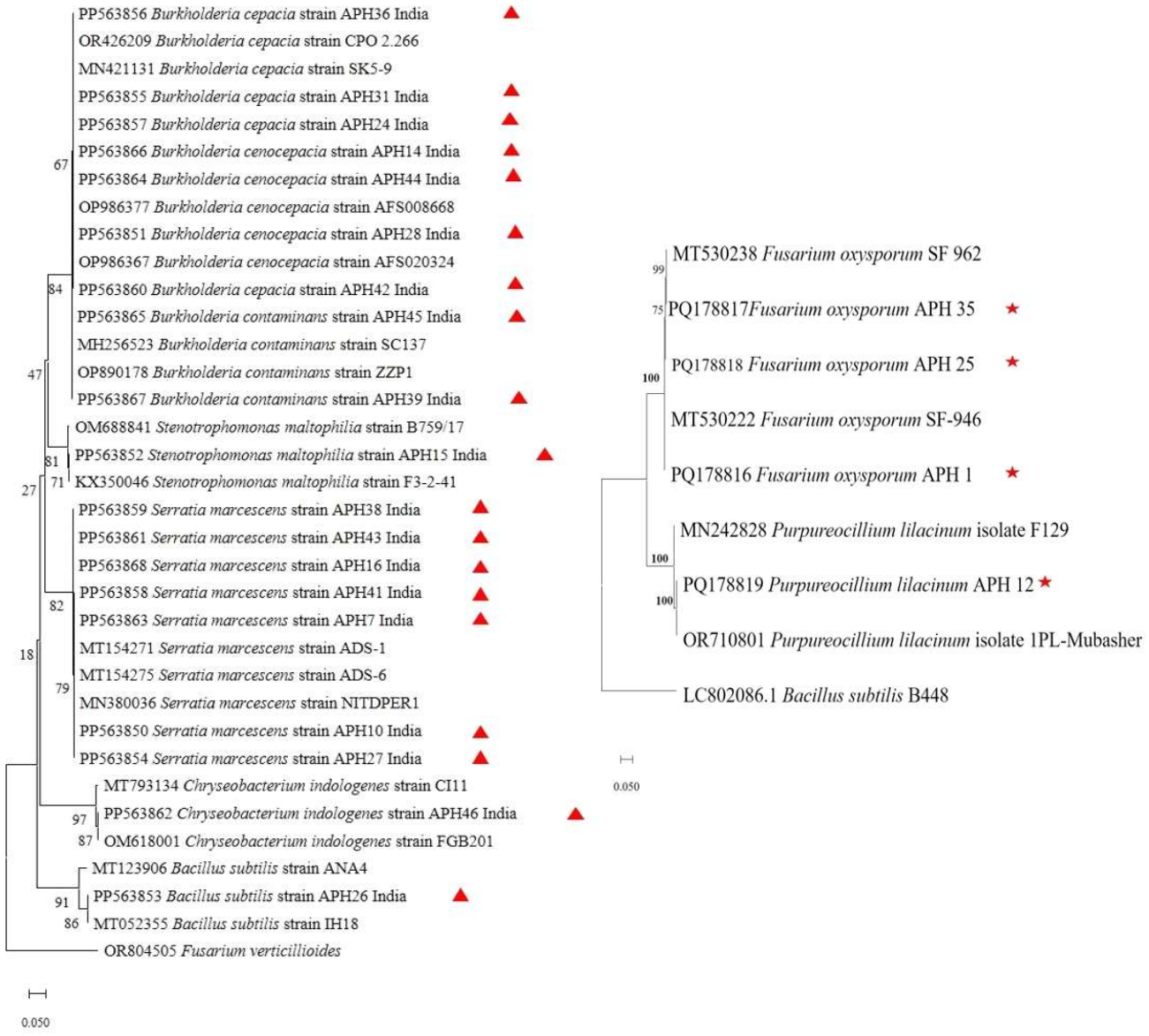


Fig. 3 : Phylogenetic analysis of all 23 isolates based on the sequences of 16s rDNA and ITS sequences using the neighbourhood joining method with 1000 bootstrap values

sequence data for closely associated strains retrieved from GenBank. The use of 16S rDNA and ITS regions in identification is well-supported in literature, where their conserved sequences among bacteria and variability among fungi provide a reliable basis for classification (Sambo *et al*., 2018). Additionally, the high resolution and efficiency of these primers in community profiling and eliminating non-target DNA have been documented in various studies (Beckers *et al*., 2016). We can better understand their roles in their respective environments by identifying specific microbial isolates.

In-vitro Screening of Bacterial and Fungal Bioagents Against *Fusarium verticillioides*

The antagonistic activity of isolated bacteria and Fungi against pathogens responsible for PFSR disease in maize was evaluated under *in vitro* conditions using the dual culture technique. The per cent inhibition of these pathogens was subsequently calculated. Two

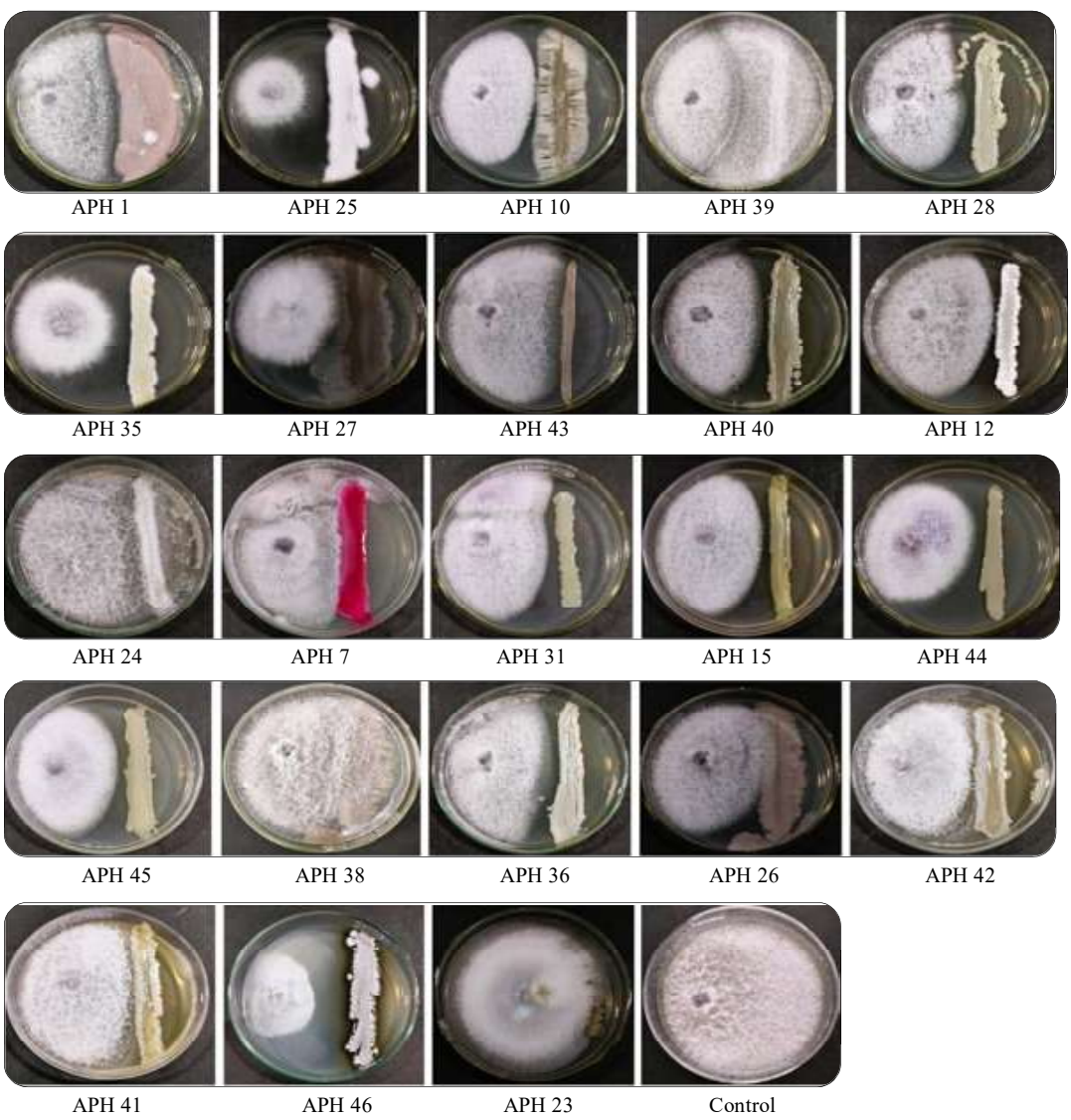
TABLE 3
NCBI GenBank accession IDs

Isolate	Organism	GenBank Accession ID
APH26	*Bacillus subtilis*	PP563850
APH28	*Burkholderia cenocepacia*	PP563851
APH44	*Burkholderia cenocepacia*	PP563852
APH14	*Burkholderia cenocepacia*	PP563853
APH31	*Burkholderia cepacia*	PP563854
APH36	*Burkholderia cepacia*	PP563855
APH24	*Burkholderia cepacia*	PP563856
APH42	*Burkholderia cepacia*	PP563857
APH45	*Burkholderia contaminans*	PP563858
APH39	*Burkholderia contaminans*	PP563859
APH46	*Chryseobacterium indologenes*	PP563860
APH10	*Serratia marcescens*	PP563861
APH27	*Serratia marcescens*	PP563862
APH41	*Serratia marcescens*	PP563863
APH43	*Serratia marcescens*	PP563864
APH7	*Serratia marcescens*	PP563865
APH16	*Serratia marcescens*	PP563866
APH38	*Serratia marcescens*	PP563867
APH15	*Stenotrophomonas maltophilia*	PP563868

isolates (APH38 and APH1) showed the highest inhibition above 50 per cent and fifteen isolates (APH35, APH29, APH43, APH27, APH7, APH28, APH45, APH41, APH46, APH10, APH14, APH23, APH31, APH36 and APH44) were showed moderate inhibition. Only five isolates showed the less inhibition (APH12, APH16, APH26, APH42 and APH24) The isolates with the highest per cent inhibition values (APH38 and APH1) (Fig. 4), these isolates stand out significantly compared to others, suggesting they may be prioritized for further studies requiring high effectiveness. *Serratia marcescens* is known for its antagonistic properties against plant pathogens, while *Burkholderia* spp. can promote plant growth and act as pathogens under certain conditions. The identification of such organisms aids in exploring their potential applications in agriculture and biotechnology. ITS-1/ITS-4 and 16S rDNA primers have proven effective in identifying a diverse range of microbial species. This methodological approach facilitates a deeper understanding of microbial communities, which is crucial for applications in microbial ecology, plant pathology and environmental biotechnology (Church *et al*., 2020).

Bacillus subtilis is a well-documented biocontrol agent known for its ability to produce a wide array of antibiotics, enzymes and secondary metabolites that inhibit the growth of various plant pathogens (Kumar *et al*., 2012). It is effective against a range of bacterial and fungal pathogens and enhances plant growth by colonizing the rhizosphere and inducing systemic resistance. Species within the *Burkholderia* genus were known for their biocontrol capabilities, particularly through the production of antibiotics and other antimicrobial compounds (Vial *et al*., 2007). *Burkholderia cenocepacia* and *Burkholderia cepacia* have shown effectiveness in suppressing soil-borne pathogens like *Fusarium oxysporum* and *Rhizoctonia solani*. *Chryseobacterium indologenes*, though less studied, has demonstrated potential in biocontrol through the production of proteolytic enzymes and antimicrobial compounds that target plant pathogens (Dahal *et al*., 2021). *Stenotrophomonas maltophilia* is recognized for its antagonistic activity against a variety of plant pathogens. It produces siderophores, proteases and antibiotics that suppress pathogen growth (Helal *et al*., 2022). *Serratia marcescens* is known for its broad-spectrum biocontrol properties, including the production of chitinases and other enzymes that degrade fungal cell walls (Kalbe *et al*., 1996). This species also produces prodigiosin, a red pigment with antifungal and antibacterial properties (Guo *et al*., 2020).

The study collected samples from diverse geographic locations, including forest soils, marine and marshy soils and river waters across Karnataka, Andhra Pradesh and Tamil Nadu, revealing a rich diversity of microbial isolates. Forest soils from western ghats harboured diverse communities supported by organic-rich environments. Coastal and marshy soils provided unique conditions like high salinity, fostering specialized microbial populations. River water samples highlighted dynamic ecosystems crucial for water quality maintenance. Molecular



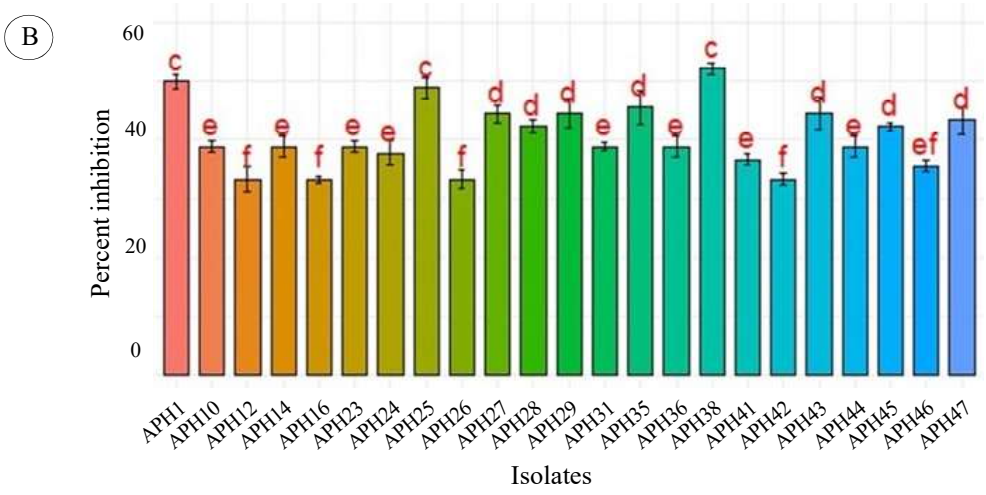


Fig. 4 : *In-vitro* screening, a) Dual plate assay for 23 isolates, b) Graph showing per cent inhibition

characterization using ITS-1/ITS-4 and 16S rDNA primers identified seven bacterial species and two fungal genera. The findings underscore the potential of these microbes in biocontrol, agriculture and environmental applications.

REFERENCES

ANONYMOUS., 2021, R Core Team.

BECKERS, B., OP DE BEECK, M., THIJS, S., TRUYENS, S., WEYENS, N., BOERJAN, W. AND VANGRONSVELD, J., 2016, Performance of 16s rDNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. *Front. Microbiol*., **7** : 650.

BERGEY, D. H., 1994, Bergey's manual of determinative bacteriology.

CHURCH, D. L., CERUTTI, L., GÜRTLER, A., GRIENER, T., ZELAZNY, A. AND EMLER, S., 2020, Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. *Clin Microbiol. Rev.,* **33** : 256 - 260.

DAHAL, R. H., CHAUDHARY, D. K., KIM, D. U., PANDEY, R. P. AND KIM, J., 2021, *Chryseobacterium antibioticum* sp. nov. with antimicrobial activity against Gram-negative bacteria, isolated from Arctic soil. *J. Antibiot*., **74** : 115 - 123.

ESMAEEL, Q., JACQUARD, C., SANCHEZ, L., CLÉMENT, C. AND AIT BARKA, E., 2020, The mode of action of plant associated Burkholderia against grey mould disease in grapevine revealed through traits and genomic analyses. *Sci. Rep*., **10** : 19393.

GUO, Z., ZHANG, X., WU, J., YU, J., XU, M., CHEN, D., ZHANG, Z., LI, X., CHI, Y. AND WAN, S., 2020, *In vitro* inhibitory effect of the bacterium *Serratia marcescens*on *Fusarium proliferatum* growth and fumonisins production. *Biological Control*., **143** : 104.

HELAL, D. S., EL-KHAWAS, H. AND ELSAYED, T. R., 2022, Molecular characterization of endophytic and ectophytic plant growth promoting bacteria isolated from tomato plants (*Solanum lycopersicum* L.) grown in different soil types. *J. Genet. Eng. & Biotechnol.,* **20** : 79.

HEO, A. Y., KOO, Y. M. AND CHOI, H. W., 2022, Biological control activity of plant growth promoting rhizo bacteria *Burkholderia contaminans* AY001 against tomato Fusarium wilt and bacterial speck diseases. *Biology*. **11** : 619.

KHALIL, M. M. R., FIERRO-CORONADO, R. A., PEŃUELAS-RUBIO, O., VILLA-LERMA, A. G., PLASCENCIA-JATOMEA, R., FÉLIX-GASTÉLUM, R. AND MALDONADO-MENDOZA, I. E., 2021, Rhizospheric bacteria as potential biocontrol agents against Fusarium wilt and crown and root rot diseases in tomato. S*audi J. Biol. Sci*., **28** (12) : 746 - 747.

KHAN, M. AND TANAKA, K., 2023, *Purpureocillium lilacinum* for plant growth promotion and biocontrol against root-knot nematodes infecting eggplant. *Plos one,* **18** : 550.

KHOKHAR, MUKESH K., HOODA, K. S., SHARMA, S. S. AND SINGH, V., 2014a, Post flowering stalk rot complex of maize-present status and future prospects. *Maydica,* **59** : 226 - 242.

KHOKHAR, M. K., SHARMA, S. S., GUPTA, R., 2014b, Integrated management of post flowering stalk rot of maize caused by *Fusarium verticillioides*. *Indian Phytopathol.,* **67** : 228 - 233.

KÖHL, J., KOLNAAR, R. AND RAVENSBERG, W. J., 2019, Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Front. Plant Sci*., **10** : 845.

KUMAR, P., DUBEY, R.C. AND MAHESHWARI, D. K., 2012, Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiol. Res*., **167** : 493 - 499.

LARENA, I., SALAZAR, O., GONZĮLEZ, V., JULIĮN, M. C. AND RUBIO, V., 1997, Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. *J. biotechnol.*, **75** (3) : 187 - 194.

PEREIRA, E. J. M. C., AMORIM, E. A. DA F., ARAGĆO, F. M. M., CĀMARA, W. DE .S., ARAŚJO, M. C., PEREIRA, C. D. DA S., DIAS, L. R. L., GOMES, W. C., ALIANĒA, A. S. DOS S., SOUZA, J. C. AND DE, S., 2023, Biocontrol

potential of *Serratia marcescens* (B8) and *Bacillus* sp. (B13) Isolated from urban mangroves in Raposa, Brazil. *Life.* **13** : 2036.

RABHI, N. E. H., SILINI, A., CHERIF-SILINI, H., YAHIAOUI, B., LEKIRED, A., ROBINEAU, M., ESMAEEL, Q., JACQUARD, C., VAILLANT-GAVEAU, N. AND CLÉMENT, C., 2018, *Pseudomonas knackmussii* MLR6, a rhizospheric strain isolated from halophyte, enhances salt tolerance in *Arabidopsis thaliana*. *J. Appl. Microbiol*., **125** : 1836 - 1851.

SAMBO, F., FINOTELLO, F., LAVEZZO, E., BARUZZO, G., MASI, G., PETA, E., FALDA, M., TOPPO, S., BARZON, L. AND DI CAMILLO, B., 2018, Optimizing PCR primers targeting the bacterial 16S ribosomal RNA gene. *BMC Bioinformatics.* **19** : 343.

SAIKAT, T. A., KHAN, M. A. S., ISLAM, M. S., TASNIM, Z. AND AHMED, S., 2024, Characterization and genome mining of *Bacillus subtilis* BDSA1 isolated from river water in Bangladesh: A promising bacterium with diverse biotechnological applications. *Heliyon*, **10** (14) : 235 - 240

SHANKARANAVAR, S. N. AND SOMASEKHARA, Y. M., 2017, *In vitro* evaluation of bioagents against *Fusarium solani* (Mart.) Sacc. causing wilt of Betelvine (*Piper betel* L.). *Mysore J. Agric. Sci.,* **51** : 621 - 624.

SHARMA, P., PANDEY, R. AND CHAUHAN, N. S., 2024, Biofertilizer and biocontrol properties of *Stenotrophomonas maltophilia* BCM emphasize its potential application for sustainable agriculture. *Front. Plant Sci*., **15** : 136.

SONG, T., GUPTA, S., SOROKIN, Y., FRENKEL, O., CYTRYN, E. AND FRIEDMAN, J., 2024, A *Burkholderia cenocepacia* like environmental isolate strongly inhibits the plant fungal pathogen *Zymoseptoria tritici*. *Appl. Environ. Microbiol*., **90** : 22 - 23.

SUDHA, S. AND NARENDRAPPA, T., 2015, *In vitro* efficacy of bioagents against *Colletotrichum gloeosporioides* causing anthracnose of mango. *Mysore J. Agric. Sci.,* **51** : 621 - 624.

TAMURA, K., STECHER, G. AND KUMAR, S., 2021, MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol*., **38** : 3022 - 3027.

WANG, B., LI, L., LIN, Y., SHEN, D., SHAO, X., ZHONG, C. AND QIAN, G., 2022, Targeted isolation of biocontrol agents from plants through phytopathogen co-culture.

WILSON, K., 1987, Preparation of genomic DNA from bacteria. *Curr. Protoc. Mol. Biol*., **5** (4) : 5 - 8.

WOESE, C. R. AND FOX, G. E., 1977, Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences*, **74** (11) : 5088 - 5090.

VIAL, L., GROLEAU, M. C., DEKIMPE, V. AND DEZIEL, E., 2007, Burkholderia diversity and versatility: an inventory of the extracellular products. *JMB*., **17** : 1407 - 1429.

VINCENT, J. M., 1947, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.***159** : 850 - 850.