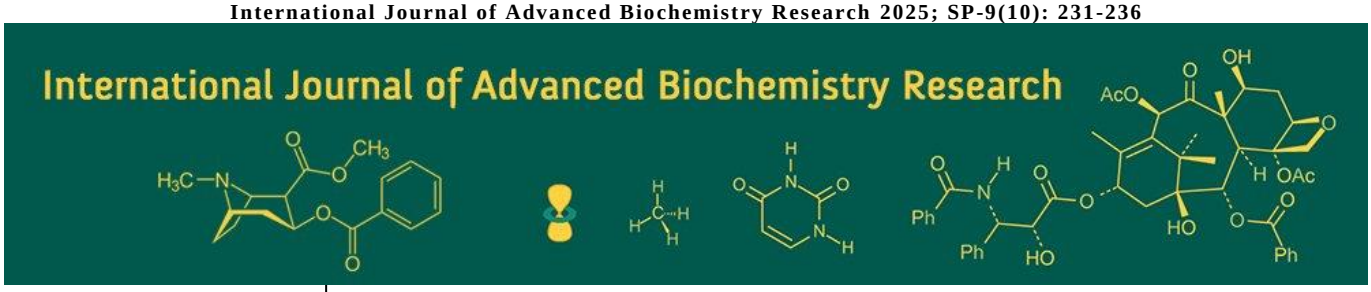

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Antagonistic and growth-promoting potential of *Stenotrophomonas* sp., an endophytic bacteria from ginger rhizomes against soft rot pathogen *Pythium myriotylum*

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Abstract
Ginger (*Zingiber officinale* Rosc.) is a major spice crop, and its cultivation is seriously constrained by soft rot disease caused primarily by *Pythium aphanidermatum* and *P. myriotylum*. In the present study, bacterial endophytes were isolated from healthy ginger rhizomes and screened for antagonistic activity against *P. myriotylum* together with plant growth-promoting traits. Four isolates showed promising disease-suppressive potential and expressed traits such as indole-3-acetic acid production, siderophore secretion, and phosphate solubilisation, confirming their dual role in growth promotion and pathogen inhibition. Among these, *Stenotrophomonas* sp. gained particular significance as this study represents the first report of its occurrence as an endophyte in ginger with detectable antagonistic activity. This novel finding broadens the understanding of the endophytic community associated with ginger and highlights its potential as a sustainable and eco-friendly alternative for the management of soft rot disease.

Keywords: Endophytic *Stenotrophomonas* sp., ginger rhizome, soft rot, *Pythium myriotylum*, antagonistic activity, growth promotion

Introduction
Ginger (*Zingiber officinale* Rosc.), a globally valued spice crop, has long been used in Ayurvedic and Tibb-Unani herbal formulations for the treatment of various ailments including indigestion, vomiting, and dementia. India is the largest producer of ginger and is known for its rich cultivar diversity, with more than 50 cultivars varying in yield and quality. Among Indian states, Kerala possesses the highest cultivar diversity, followed by the northeastern regions (Ravindran *et al*., 2005) [19].

Despite its economic and medicinal importance, ginger cultivation is severely constrained by several diseases, particularly those that are soil-borne and rhizome-borne, such as soft rot, yellows, and bacterial wilt. Of these, soft rot disease caused by *Pythium aphanidermatum* is the most destructive, leading to yield losses ranging from 70% to 90% during a cropping season. Earlier studies reported up to 11 *Pythium* species associated with soft rot, with *P. aphanidermatum* and *P. myriotylum* identified as the most prevalent and devastating (Dohroo, 2005) [5]. The disease is widespread across all major ginger-growing regions of India, particularly in Kerala, where losses can reach as high as 80% in exceptionally wet years (Ramakrishnan, 1949) [17].

Chemical fungicides have been used to suppress the disease, but their application is neither sustainable nor cost-effective in the long term (Gangawane and Shaik, 1988) [7]. Additionally, fungicide use raises concerns regarding residue accumulation, soil pollution, and pathogen resistance. These limitations highlight the urgent need for eco-friendly and sustainable alternatives for disease management.

Endophytes represent a promising solution, as they colonize internal plant tissues without causing visible harm to the host (Stone *et al*., 2000) [23]. Their role in natural biocontrol has attracted attention due to their co-evolution with plants, persistence within host tissues, and strong antagonistic activity against host-specific phytopathogens (Brum *et al*., 2012) [2].

Endophytic bacteria suppress pathogens either directly through the production of antimicrobial metabolites, siderophores, and hydrolytic enzymes (Wang *et al*., 2010) [26] or indirectly by inducing systemic resistance in host plants.

In this context, the present study was undertaken to isolate and characterize bacterial endophytes from ginger rhizomes, evaluate their antagonistic potential against the soft rot pathogen *Pythium myriotylum*, and assess their plant growth-promoting traits. Notably, this study also highlights the antagonistic potential of *Stenotrophomonas* sp. as an endophyte, adding new insights into its biocontrol role in ginger.

Materials and Methods

Isolation of the soft rot pathogen

The isolation of the soft rot pathogen was carried out by the tissue segment method described by Rangaswamy (1958) [18]. Infected samples were washed under running tap water and cut into small segments along with adjacent healthy tissue. The segments were surface sterilized with 1% sodium hypochlorite for 1 min, rinsed three times in sterile distilled water, and blotted dry on sterile paper. Sterilized bits were placed on potato dextrose agar (PDA) amended with antibiotics and incubated at room temperature for 2 days. Fungal growth was sub-cultured by aseptically transferring hyphal tips to fresh PDA to obtain pure cultures.

Collection of samples and isolation of endophytic microbes

Healthy ginger rhizomes were purposively collected from AEU 10 and AEU 15 of Thrissur district. Endophytic bacteria were isolated following the surface sterilization method of Schulz *et al*. (1993) [20] with slight modifications. Rhizome segments (1-2 cm) were washed under running tap water, treated with Tween-80 (10 min), and rinsed with distilled water. Surface sterilization was performed sequentially with 70% ethanol (1 min), sodium hypochlorite (4% available chlorine, 5 min), and 70% ethanol (30 s), with sterile distilled water washes between treatments. Sterilized rhizomes were dried on sterile filter paper, rinsed with phosphate-buffered saline (PBS, pH 7), and sterility was confirmed by plating both the final water wash and PBS rinse on nutrient agar. The rhizome pieces were macerated in 9 ml of phosphate-buffered saline (PBS) and serial dilutions were prepared up to 10^{-6}. Aliquots (1 ml) from the appropriate dilutions were pour-plated on nutrient agar (NA). The plates were incubated at 28±2 °C for 48 h.

In vitro assessment of the bacterial endophytes against *Pythium myriotylum*

The isolated bacterial endophytes were tested for their *in vitro* antagonistic effect against the pathogen by dual culture method (Utkhede and Rahe, 1983) [24]. Mycelial disc of the pathogen was placed at the center of PDA plates, while the bacterial endophytes were streaked on opposite sides of the pathogen 1 cm from the plate edge. Three replications were maintained for each isolate, along with a control. Plates were incubated at room temperature until the control plate reached full growth. percent inhibition (PI) of pathogen growth was calculated using the standard formula (Vincent, 1927) [25].

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

where C is the growth of pathogen in control plate and T, the growth of pathogen in treatment plate.

Biocontrol potential and growth promotion of the of bacterial endophytes

The biocontrol potential and growth promotion activity of the selected antagonistic bacterial endophytes were studied by estimating the production of volatile metabolites (Dennis and Webster, 1971a) [4], siderophore (Kloepper *et al*., 1980) [16], indole acetic acid (IAA) (Gordon and Weber, 1951) [11], and phosphate solubilisation (Gupta *et al*., 1994) [12] adopting standard protocols.

Molecular identification

The samples of the promising bacterial isolates were outsourced to Csix Labs Pvt. Ltd., Palakkad, for molecular analysis. Bacterial DNA was extracted using the QIAGEN DNeasy UltraClean Microbial Kit (Cat. No./ID: 12224-50), and the quality of the genomic DNA was confirmed on a 1.0% agarose gel, which showed a single high-molecular-weight band. The 16S rRNA gene fragment was amplified using 16SrRNA-F and 16SrRNA-R primers under the following PCR conditions: initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s; followed by a final extension at 72 °C for 3 min. A single discrete amplicon was obtained and purified using the QIAGEN QIAquick PCR Purification Kit (Cat. No./ID: 28104). Sequencing of the purified PCR product was performed in both directions using the same primers with the BDT v3.1 Cycle Sequencing Kit on an Applied Biosystems™ MiniAmp™ Plus Thermal Cycler, and the products were analysed on an ABI 3730xl Genetic Analyzer.

Results and Discussion

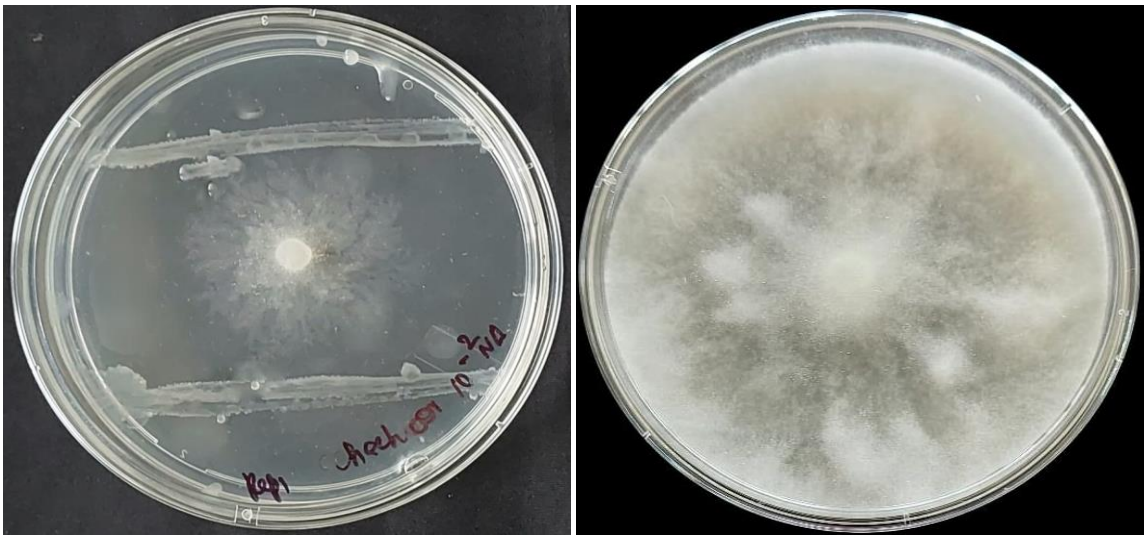
Ginger is widely cultivated as a spice crop; however, its rhizomes are highly susceptible to various biotic stresses, among which soft rot is considered the most destructive. In the present study, the pathogen associated with soft rot of ginger was identified as *Pythium myriotylum*, consistent with earlier reports describing this species as the predominant causal agent of soft rot in ginger-growing regions. The aggressive nature of *P. myriotylum* and its ability to survive under diverse environmental conditions make management of the disease particularly challenging. Endophytic microorganisms have gained considerable attention as promising alternatives for sustainable disease management, owing to their ability to colonize internal plant tissues and confer protection against pathogens.

In the present study, a total of 27 bacterial endophytes were isolated from the healthy ginger rhizomes, after rigorous surface sterilization and sterility checks, ensuring the absence of epiphytic contamination. The isolates were coded with a naming scheme, the letter “G” represented the crop (ginger), subsequent letters denoted the location of collection, and “B” indicated that the isolate belonged to bacteria.

In vitro dual culture assays revealed that four bacterial isolates exhibited strong antagonistic activity against *P. myriotylum*, with percent inhibition ranging from 68.51 to 74.44 (Table 1). Notably, *Stenotrophomonas* sp. was recorded for the first time as an endophyte in ginger showing antagonistic activity, with 68.51% inhibition (Fig. 1). The biocontrol potential of all these endophytic bacterial

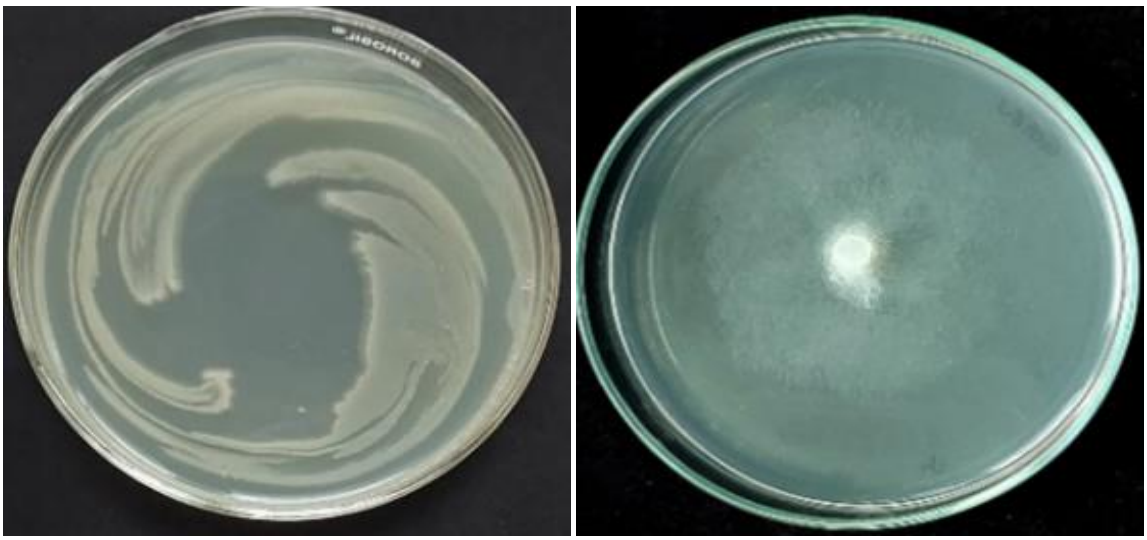
isolates was further evaluated (Table 2) by assessing their ability to produce volatile organic compounds (VOCs) by sealed Petri plate technique. In this study, VOC production was detected only in one bacterial endophyte (GChB-3) restricting the pathogen's growth to a limited extent, while no other isolates produced detectable volatile metabolites (Fig 2). Qualitative analysis of siderophore production on chrome azurol S (CAS) agar showed that all the four isolates were capable of producing siderophores, as indicated by the appearance of a greenish-yellow fluorescent pigment (Fig 3). The supernatant of the endophytic isolates reacted positively with Salkowski's reagent, confirming that all isolates produced indole-3-acetic acid (IAA), as indicated by a colour change from the control after 30 minutes of incubation (Fig 4). Phosphate-solubilizing activity was assessed on Pikovskaya's agar, where bacterial secretion of organic acids dissolves insoluble tricalcium phosphate, producing clear halo zones around colonies. All isolates demonstrated phosphate-solubilizing activity, with GVB-9 showing the strongest capability, while other isolates produced only minimal halos (Fig 5). Molecular characterisation of the selected bacterial isolates was carried out through 16S rRNA sequencing and the organisms were identified as *Pseudomonas aeruginosa* (GVB-9), *Bacillus* sp. (GCB-7), *Bacillus* sp. (GPuB-5), and *Stenotrophomonas* sp. (GChB-3).

The antagonistic and growth promotion activity of the bacterial endophytes viz., *Pseudomonas aeruginosa* and *Bacillus* sp. has already been reported (Alström, 2001; Wheatley, 2002) [1, 27]. Kai *et al*. (2006) [13] demonstrated that small volatile organic compounds released by bacterial antagonists negatively affected the mycelial growth of the soil-borne phytopathogen *Rhizoctonia solani* Kühn. No similar studies have been carried out with regard to bacterial endophyte, *Stenotrophomonas* sp. In the present study, with respect to volatile organic compound production, *Stenotrophomonas* sp. was the only endophyte which showed the production of volatiles inhibiting the pathogen growth while no other isolates produced detectable volatile metabolites. Although the inhibition was minimal, this finding provides novel insight, as it is the first report highlighting the endophytic antagonistic potential of *Stenotrophomonas* in ginger and its ability to produce inhibitory volatiles, even at a limited level.



A B

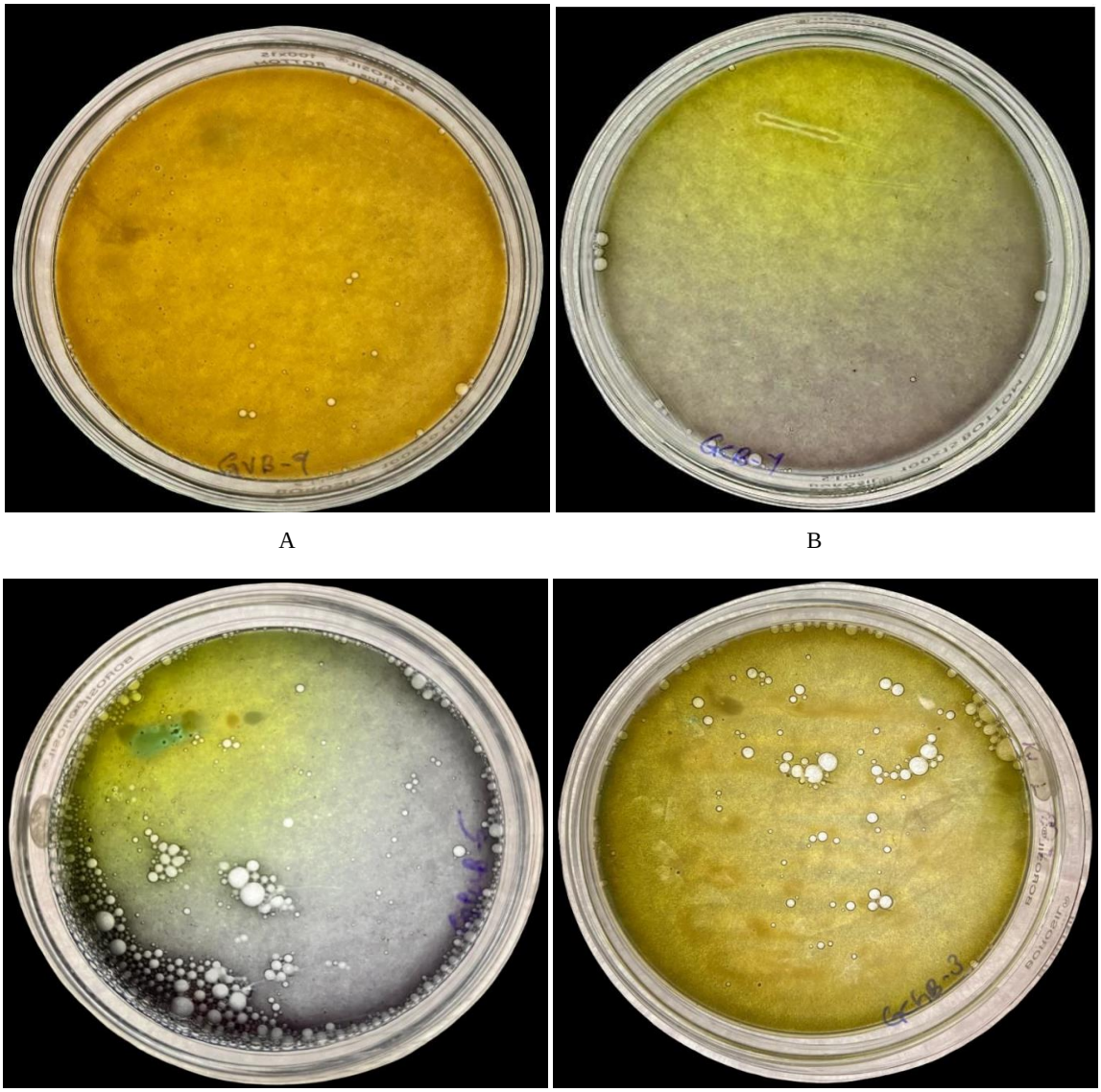
Fig 1: Inhibition of *P. myriotylum* by the isolate GChB-3 (*Stenotrophomonas* sp.) by dual culture technique



A B

Fig 2: Production of volatile metabolites by sealed Petri plate technique

All the four bacterial isolates including *Stenotrophomonas* showed siderophore production on chrome azurol S (CAS) agar indicated by the appearance of a greenish-yellow fluorescent pigment. Siderophore-producing bacteria are known to sequester iron, inhibiting pathogen growth while also promoting plant development, thereby enhancing their overall effectiveness as biological control agents (Spadaro, 2016; Numan *et al*., 2018) [22,16]. Various strains of *Bacillus* spp. and *Pseudomonas* spp. have demonstrated plant growth-promoting and pathogen-suppressing activities (Cherif-Silini *et al*., 2016; Gao *et al*., 2022) [3,8]. Although endophytic *Stenotrophomonas* from ginger rhizomes has not been previously reported for its biocontrol potential, it is known to produce siderophores that contribute to plant growth promotion (Jasim *et al*., 2014) [6]. The rhizospheric isolate *Stenotrophomonas chelatiphaga* has been reported to enhance plant growth and iron uptake in canola and maize, highlighting the biocontrol and growth-promoting potential of siderophore-producing rhizobacteria (Ghavami *et al*., 2017) [9].



A B C D

Fig 3: Production of siderophore by promising isolates

All the four bacterial endophytes produced indole-3-acetic acid (IAA), as indicated by a colour change from the control after 30 minutes of incubation. Endophytes are recognized for producing phytohormones such as IAA, which play a pivotal role in plant growth and development while enhancing tolerance to environmental stresses. Although many rhizospheric bacteria are known to synthesize IAA, studies focusing on endophytic IAA production in ginger are limited, highlighting the relevance of the present investigation and suggesting that these isolates including *Stenotrophomonas* could significantly contribute to plant growth promotion through phytohormone production.

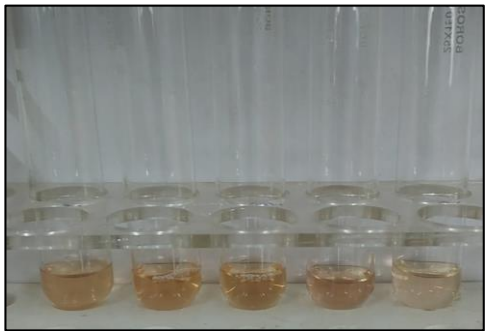
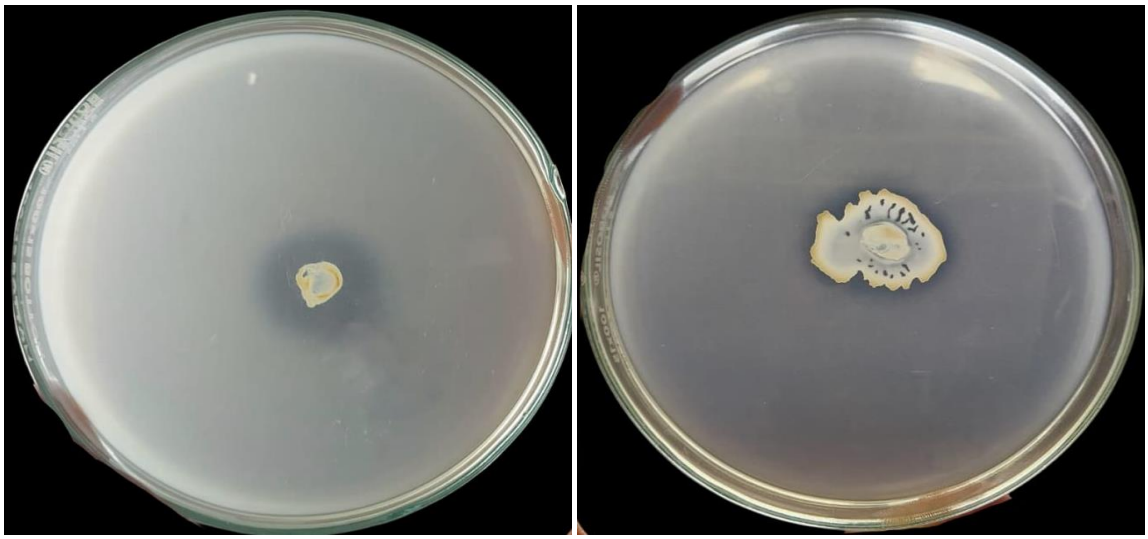


Fig 4: Indole acetic acid production

Microorganisms also play a key role in enhancing phosphorus bioavailability by solubilizing inorganic compounds, making phosphorus accessible for plant uptake. It was observed from the study that all the bacterial isolates demonstrated phosphate-solubilizing activity. Reports on phosphate-solubilizing endophytic *Stenotrophomonas* from ginger rhizomes are limited, highlighting the novelty of our findings. Most phosphate-solubilizing *Stenotrophomonas* strains have been isolated from rhizospheric soils or mines (Xiao *et al*., 2009; Sharma *et al*., 2025) [28, 21]. Numerous studies on both rhizospheric and endophytic *Pseudomonas* and *Bacillus* have already been reported on their phosphate-solubilizing capability (Mei *et al*., 2021; Gupta *et al*., 2022) [15, 12].



A B

Fig 5: Phosphate solubilisation of the antagonistic isolates

Conclusion

The present study demonstrates that ginger rhizomes harbour a diverse community of endophytic bacteria with potential roles in plant health. All the isolated bacterial endophytes exhibited biocontrol potential along with plant growth-promoting traits, including indole-3-acetic acid (IAA) production, siderophore secretion, and phosphate solubilization. The findings underscore the dual role of ginger-associated endophytes in both disease suppression and plant growth promotion, offering a sustainable and eco-friendly approach to managing soft rot disease in ginger cultivation. Among the endophytes, *Stenotrophomonas* sp. (GChB-3) emerged as a noteworthy antagonist against *Pythium myriotylum*, marking the first report of its antagonistic activity as an endophyte in ginger, although its efficacy as a non-endophytic biocontrol agent has been documented previously.

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