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Research Article

Biocontrol ability of indigenous *Pseudomonas* sp.VSMKU4040 against economically important fungal phytopathogens for the sustainable agriculture

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ABSTRACT: Crop activation relies completely on the rhizosphere's bacteria. In this instance, we looked into the effectiveness of *Pseudomonas* spp. present in the root zone of tomato against various plant diseases like *Rhizoctonia solani, Sclerotium rolfsii,* and *Fusarium oxysporum* RACE, which range from 12.33mm to 21.66mm in the Zone of Inhibition (ZOI). Interestingly, all five *Pseudomonas* spp. significantly produced antimicrobial and plant growth-promoting traits involved in plant development, including siderophore, hydrogen cyanide, IAA, and phosphate solubilization. Furthermore, the biocontrol mechanism of parasitism was found to be considerably influenced by the secretion of hydrolytic enzymes by *Pseudomonas* spp., including chitinase, cellulase, protease, and amylase. The particular strain designated as VSMKU4040 was selected for further study out of five strains of *Pseudomonas* spp. because of its outstanding antagonism and other unique biological control mechanism properties. The cell-free culture supernatant of *Pseudomonas* spp. effectively suppressed almost all of the fungal plant diseases indicated above, compared to the control. Pinch of *R. solani* and *S. rolfsii* mycelia from the ZOI during interaction with a *Pseudomonas* spp. VSMKU4040 was processed for examination under a scanning electron microscope. In comparison to the control, we discover that *R. solani* mycelium broke, disintegrated, and deformed. In contrast, during *Pseudomonas* spp. VSMKU4040 interaction with *S. rolfsii*, there was no initiation of sclerotia development along with *Pseudomonas* spp. VSMKU4040 distribution, whereas in the control, we could observe the formation and intimation of sclerotia bodies in *S. rolfsii*, in comparison to the control. When the 16s rRNA encoding gene was compared to a 1kb marker, 1500 bp was found in *Pseudomonas* spp. VSMKU4040. The pure ethyl acetate extract yielded three distinct metabolites, each with a different Rf value (0.91, 0.85, and 0.8) that were found in Thin-Layer Chromatography (TLC). By using the good diffusion method, the second molecule (0.85) out of the three showed significant potential activity against fungal plant pathogens at 30µg/ml when compared to a commercial fungicide companion.

KEYWORDS: Fungal phytopathogens, metabolites and antifungal activity, *Pseudomonas* spp.

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INTRODUCTION

A popular vegetable around the world, the tomato (*Solanum lycopersicum* L.) is essential to local and national food security as well as socioeconomic development (Liu *et al.*, 2022; Cen *et al.*, 2022; Suresh *et al.*, 2022a). This well-known solanaceous crop gained notoriety in India during the Red Revolution. However, there are several obstacles to tomato production, both biotic and abiotic. Among the biotic stressors, *R. solani* and *S. rolfsii* caused damping-off disease poses a serious threat to tomato farming and can have disastrous consequences (Artal *et al.*, 2012; Nion & Toyota, 2015). Fungal infections In the greenhouse and open field environments, *R. solani* and *S. rolfsii* regularly induce damping-off on several horticultural crops, particularly tomatoes. This results in an impairment of productivity. (Berta *et al.*, 2005). With output losses reported to range from 35% to 90% in India alone, this disease has become a major obstacle for tomato growers globally. According to Shanmugaiah *et al.*, (2010), is considered the second-most important fungal plant pathogen globally, ranking behind *R. solani* and *S. rolfsii* as far as both scientific and economic importance.

Damping off is a dangerous disease, as this pathogen infects tissues even in their early stages of germination and causes even more severe disease in plants. Several plants, including tomatoes (*S. lycopersicum* L.), are susceptible to damping off disease, which impacts the plant in nursery beds at both the pre-and post-emergence stages. Infected

seeds show signs of pre-emergence damping off before they germinate (Desalegn *et al*., 2021). This can cause the seed to decay or turn brown, which is indicative of poor or no germination. Due to the extensive application and buildup of chemical ingredients in agriculture fields, our environment is highly contaminated. In this context, agriculture practitioners may use chemical fungicides and fertilizers to control those economically significant infections. In addition, there is a greater greenhouse gas emission and temperature. Therefore, we must develop a different strategy to control harmful pathogens in agricultural fields.

Researchers have recently hard-pressed for the development of fresh, practical approaches in managing soil-borne infections. New strategies for controlling plant diseases include biological and combined control that uses antagonistic microbes alone, in combinations, or alternately with fungicides or organic adjuvants (Shanmugaiah *et al.*, 2008). Several investigations have exposed that an array of useful microorganisms for plants, which are discovered in the plant's rhizosphere, can protect plants from soil-borne bacterial and fungal diseases. Examples of these microorganisms are *Pseudomonas* species, (Qessaoui *et al*., 2019, Nithya *et al.*, 2020), *Bacillus* species (Shanmugaiah *et al.*, 2008), *Trichoderma* species (Shanmugaiah *et al.*, 2009), and *Streptomyce*s species (Harikrishnan *et al.*, 2016; Ebrahimi-Zarandi *et al.*, 2021).

This study aimed to examine how phyto fungal plant pathogens are managed by using the tomato rhizosphere bacterium *Pseudomonas* spp. VSMKU4040. The *Pseudomonas* spp. VSMKU4040 ability to produce antibacterial and growth-promoting characteristics were evaluated with partially purified potential molecule from *Pseudomonas* spp. VSMKU4040 against soil-borne pathogens.

METHODOLOGY

Procurement of *Pseudomonas* spp. and fungal plant pathogens and their maintenance

The Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai – 625 021, Tamil Nadu, India, provided five *Pseudomonas* species. All of the isolates in our collection, previously isolated, described, and determined as *Pseudomonas* spp. by biochemical analysis, were given accession numbers that began with the five-letter VSMKU and ended with a numerical number. The isolates were kept in 30% sterilized glycerol stock solution at 80°C. In this work, the test pathogens were *R. solani, F. oxysporum, S. rolfsii,* and *F. oxysporum* RACE4 which are damping-off and wilt pathogens. For frequent usage, they were preserved in test tubes with Potato Dextrose Agar (PDA) at 4°C.

Antagonistic activity of *Pseudomonas* spp. against phytofungal plant pathogens

Using the dual plate assay method, all five isolates were tested in triplicate on PDA medium against phyto fungal pathogens, including *R. solani, F. oxysporum, S. rolfsii,* and *F. oxysporum* RACE4 (Gomathinayagam *et al.,* 2012). Using a sterile cork borer, nine-millimeter mycelia discs were removed from the fungus's young developing edge and positioned in the Petri dish's center. The PDA plate was aseptically infused with 24 bacterial isolates via punctiform inoculation, and the incubation period was 5 days at 28±2°C. Three duplicates were retained for each isolate. The inhibition zone between the two cultures was measured to the closest millimeter compared to the control.

Interaction of fungal pathogens and *Pseudomonas* spp. VSMKU4040 visualized under scanning electron microscope

Rhizoctonia solani and *S. rolfsii* fungal mycelium that were moving towards the interaction zone were prepared for SEM examination. Agar discs with a thickness of 1 mm were taken from the interaction zone and positioned on cover glasses. These were left at 20°C for 24 h with 2% osmium tetroxide exposure. The samples were placed on copper stubs using gold-coated double adhesive tape in a POLARON, AU/PD sputter coater. They were then scanned at 30 Kv in a Philips 505, Holland SEM and the prospect of any anomalies in the hyphae of *R. solani* and *S. rolfsii* were noted at a 1000-times magnification (Shanmugaiah, 2007).

Production of hydrolytic enzymes

The qualitative assay methodology was used to investigate the synthesis of hydrolytic enzymes in all five antagonistic isolates of FPs (Varatharaju *et al.*, 2020). For all the isolates of FPs on nutritional agar enriched with 1% of the corresponding substrates, the hydrolytic enzyme manufacturing test was used to measure the formation of chitinase, protease, amylase, cellulase, and pectinase (Shanmugaiah *et al.*, 2008). After 24 h of incubation, hydrolytic enzyme activity was seen in a clear zone surrounding the FP cultures as opposed to the control.

Pseudomonas spp. produces antibacterial characteristics and plant growth-promoting substances

Indole Acetic Acid (IAA) production

The production of indole acetic acid by all five of the FP isolates was evaluated using the method of Kalimuthu *et al.* (2019). The FPs were inoculated with 0.4% L-tryptophan supplementation in King's B broth and left to incubate for a full day. Four millilitres of Salkowski reagent (1 millilitre of 0.5 M $FeCl_3$ in 50 millilitres of 35% $HClO_4$) and two millilitres of cell-free culture filtrate were added after a

15 min centrifugation at 10,000 rpm, and the mixture was incubated for 20 min at a moderate temperature.

Phosphate solubilisation

With a few minor adjustments, all five of the FP isolates were inoculated with punctiform on Pikovskaya's agar (Suresh *et al.*, 2022). For 48 h, the plates were incubated at 28±2°C. It was believed that the development of a clear zone around the colony was advantageous for the solubilization of insoluble phosphate in comparison to the control.

Siderophore production

Using the methodology of Shanmugaiah *et al.* (2006), the identities of all five FP isolates were ascertained using the Chrome Azurole S (CAS) assay. The FPs were injected into cetrimide agar after a 24 h incubation period. After incubation, the plates were incubated with 0.7% CAS agar on top of cetrimide agar for 24 h at 28°C. A change in colour from blue to brownish-orange was indicated by the emergence of siderophores.

Morphology, biochemical and 16srDNA analysis

The selected isolate VSMKU4040 was identified by colony morphology, and cell shape, including gram staining and pigmentation. The pyocyanin pigment was observed on King's B medium. Isolate VSMKU4040 was observed as a rod shape under a light microscope by staining it with a Grams reaction. Bergey's Manual of Determinative Bacteriology performed biochemical analysis. In addition, the strain VSMKU4040 was chosen for additional research based on its highest antagonistic performance. Universal primers were used to amplify the 16S rDNA gene. 1492R (50 TACGGCTACCTTGTACGACTTCACCCC) and 27F (50 AGAGTTTGATCCTGG-TCAGAACGCT) (Nithya *et al.*, 2019).

Detection of metabolites on TLC

The ethyl acetate extracts were air-dried at 50°C using a rotary evaporator. The crude extracts of *Pseudomonas* spp. VSMKU4040 were examined using TLC. An ethyl acetate reagent (Sigma Aldrich, Mumbai) was used to observe 1 mg of crude extracts from *Pseudomonas* spp. VSMKU4040 FPs on silica gel plates (Merck, Germany). Acetic acid and benzene were used in a 4:1 ratio to create the TLC plates (Nithya *et al.*, 2020). The spots were found at 254 nm under UV light, and the Rf values of the crude metabolites were calculated.

Antifungal activity of crude metabolites from *Pseudomonas* spp. VSMKU4040 against phyto fungal plant pathogens

After dissolving the concentrated crude extract in ethyl acetate, 30µg/ml, or 25µl to 100µl, was applied to each PDA plate well. Mycelial discs (9 mm in diameter) with five-day-old fungal pathogens (*R. solani, S. rolfsii, F. oxysporum,* and *F. oxysporum* RACE) were located in the middle of the PDA plates. After that, the plates were incubated at 28°C (Mathivanan & Shanmugaiah, 2011). Three identical plates were created for each treatment. The mycelial growth of the fungus was seen after three to five days.

RESULTS AND DISCUSSION

Antagonistic activity of *Pseudomonas* spp. against phytofungal plant pathogens

The antifungal characteristics of *Pseudomonas* species were assessed *in vitro* using dual culture techniques. Maximum mycelial growth inhibition (21.66, 20.67, 16.33, and 18.33 mm) was seen in the isolate VSMKU4040 against fungal pathogens, including *R. solani, S. rolfsii, F. oxysporum* RACE*,* and *F. oxysporum*. In comparison to the control indicated in Table 1, the minimum expansion of the inhibition zone was observed for other isolates of *Pseudomonas* spp. such as VSMKU4012, VSMKU4020, VSMKU4023, and VSMKU4028 against comparable pathogens with varying levels of the zone of inhibition (Figure 1a-d). Due to their highly antagonistic modification towards the exterior and interior tissues of leaves, roots, and stems, rhizosphere-associated luminous pseudomonads (FPs) have recently been considered an important type of helpful microbes for controlling soil-dwelling bacterial and fungal plant pathogens (McGovern, 2015, Suresh *et al.*, 2022a). Studies have shown that fluorescent pseudomonads are mostly present in the rhizosphere of crop plants (Shanmugaiah *et al.*, 2006). Many plant diseases can be effectively combatted by *Pseudomonas* species. Research on *Pseudomonas* spp. treated against fungal pathogens suggests that FPs may have broad-ranging activity towards bacterial and fungal soil-borne pathogens when they interact with plant pathogens and release various antibiotics, hydrolytic enzymes, volatile compounds, and plant growth hormones.

Interaction of *Pseudomonas* spp. VSMKU4040 and fungal phytopathogens visualized under scanning electron microscope

Pseudomonas spp. VSMKU4040's outstanding antagonistic activity against several fungal plant diseases led to the decision to carry out more research on the organism. A scanning electron microscope was used to identify the antagonistic connection between test *Pseudomonas* spp. VSMKU4040 and fungal infections such as *R. solani* and *S. rolfsii*. The control plate's micrographs showed that the hyphal structures of the fungus were unaltered and unharmed, but the interaction between the fungi and the antagonistic isolate VSMKU4040 showed that the bacteria had attached to the fungus mycelial structure. In a dual culture of *Pseudomonas* spp. VSMKU404 and *R. solani*, discontinuity of mycelium, disintegration, deterioration, atrophy, and lysis of *R. solani*

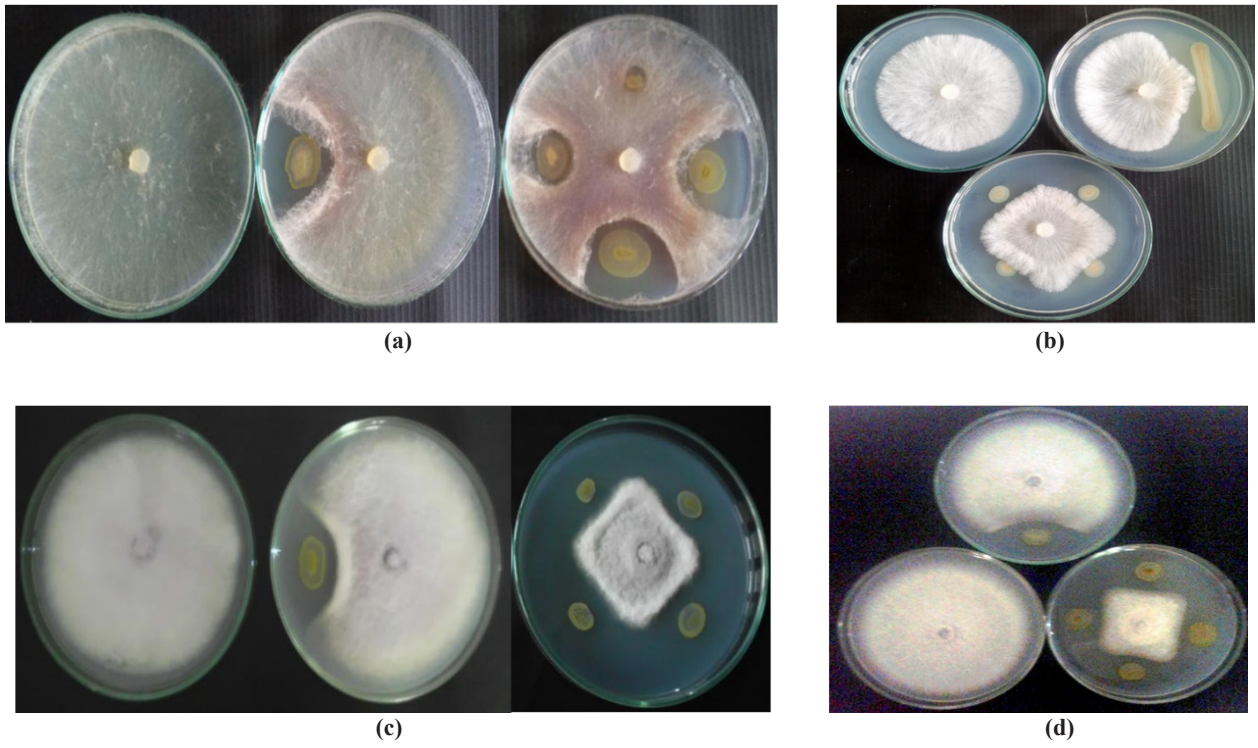


Figure 1. Antagonistic activity of FPs against fungal phyto pathogens: **(a)** *R. solani* + FPs, **(b)** F. *oxysporum*, **(c)** *S. rolfsii* + FPs, **(d)** *F. oxysporum* RACE + FPs.

Table 1. Antagonistic activity of Fluorescent Pseudomonads (FPs) against fungal phytopathogen

FP`s isolates	Zone of growth inhibition (mm)			
	R. solani	***S. rolfsii***	***F. oxysporum* RACE**	***Fusarium oxysporum***
VSMKU4012	12.33±2.52	14.00±1.73	15.00±1.00	15.67±0.58
VSMKU4020	13.00±1.00	13.00±0.00	14.33±1.15	18.00±0.00
VSMKU4023	15.00±1.00	12.33±2.08	16.00±1.00	16.33±0.58
VSMKU4028	7 ± 1.00	15.00±1.73	14.00±1.00	18.33±0.58
VSMGT 4040	21.66±0.58	20.67±0.58	16.33±1.53	18.33±2.08

mycelium were observed under a Scanning Electron Microscope (SEM) (Figure 2a). Under SEM observations in the dual plate of *Pseudomonas* spp. VSMKU4040 and *S. rolfsii*, the case test plate showed disintegration, pulsing, breaking, deformities, and accumulation of the rod-shaped antagonistic isolate *Pseudomonas* VSMKU4040. On the other hand, the control showed no alterations (Figure 2b). We found that the hyphae of *R. solani* f. spp. *sasakii* mycelium from a dual culture plate experiment were significantly distorted when viewed under a microscope in comparison to the control as in Figure 2c and d. The isolates of *Pseudomonas* species such as AS19 and AS21 caused the hyphae of *R. solani* f. spp. *sasakii* to become lysed and distorted. Furthermore, both have the ability to significantly alter the fungus's hyphal shape. As stated by Tang *et al.*, (2024).

In recent decades, biological control systems have piqued the curiosity of numerous academics. Bio agents pose a direct threat to pathogens through the manufacturing of antibiotics, lytic enzymes, and hyperparasitism; they also pose an indirect threat by competing for nutrients and space, causing systemic resistance, and plant growth promotion (Velivelli *et al.*, 2014). The majority of the researchers used a scanning electron microscope and a light microscope to find morphological anomalies like atrophy and lysis using *P. polymyxa* in both fungal mycelia, as well as overgrowth and lysis of *F. oxysporum* f. spp. *capsici* and *R. solani* in dual cultures with *T. longibrachiatum* and *Streptomyces aurantiogriseus* VSMGT1014. *Trichoderma* species have been shown to parasitize pathogenic fungi in other investigations. *Trichoderma harzianum* strains are

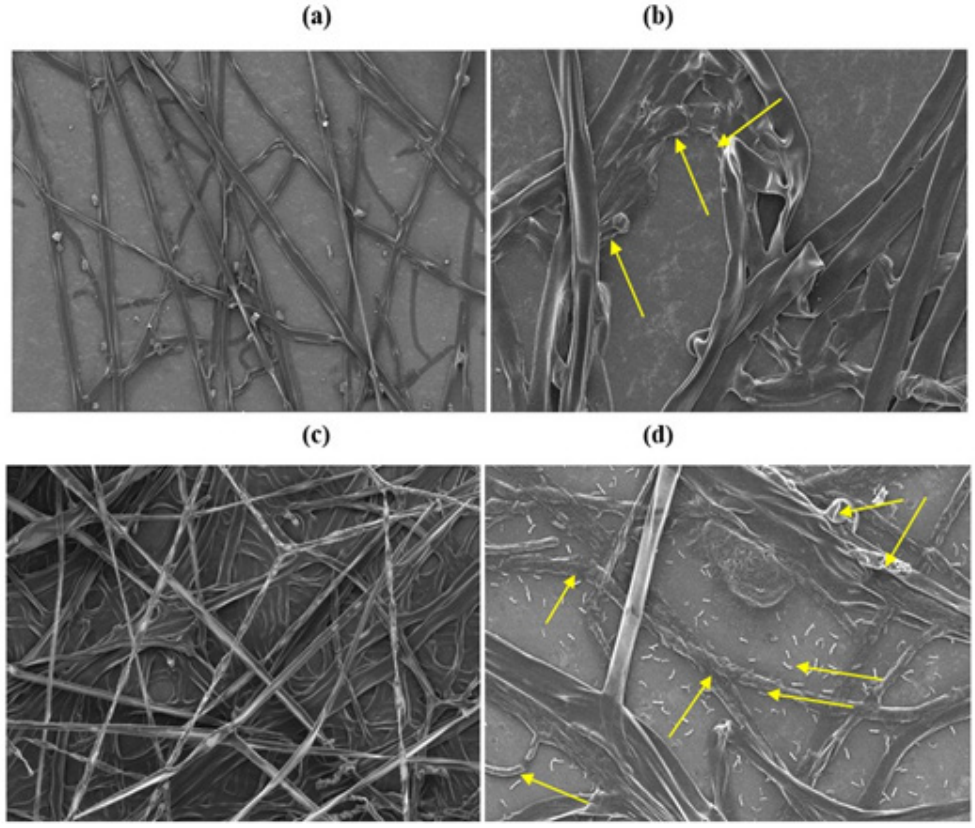


Figure 2. Antagonistic interaction between *Pseudomonas* spp. VSMKU4040 against phyto fungal pathogens visualized under SEM (**(a)** *R. solani*, **(b)** *R. solani* + *Pseudomonas* spp. VSMKU4040, **(c)** *S. rolfsii*, **(d)** *S. rolfsii* + *Pseudomonas* spp. VSMKU4040). Pathogenic fungal (*R. solani* and *S. rofsii*) mycelial changes were indicated with arrows**.**

Table 2. Plant-growth promoting and antimicrobial traits from isolates of *Pseudomonas* spp.

Isolates of FPs	Protease	Chitinase	Amylase	Cellulase	IAA	Siderophore	Phosphate solubilization
VSMKU4031	+	+	+	+	+	+	+
VSMKU 4036	+	+	+	+	+	+	+
VSMKU4038	+	+	+	+	+	+	+
VSMKU4028	+	+	+	+	+	+	+
VSMKU 4040	+	+	+	+	+	+	+

antagonistic with *R. solani*, according to scanning electron microscopy research (Harikrishnan *et al.*, 2014). The *T. harzianum* Th-9 isolate grew and coiled around the *R. solani* cells, invading and damaging the host hypha. The mechanical activity allows for the penetration of the host cells. It has been discovered that the secretion of antifungal chemicals inhibits the growth of many plant diseases (Hassan *et al.*, 2024).

Functional characterization of *Pseudomonas* spp. VSMKU4040 against fungal plant pathogens

Pseudomonas species exhibited a notable degree of mycelial inhibition *in vitro* antagonistic activity against phyto fungal infections. Subsequently, these strains underwent screening for the synthesis of hydrolytic enzymes, characteristics that promote plant growth, and antifungal metabolites. It's interesting to note that hydrolytic enzymes like protease, chitinase, amylase, and cellulase were significantly produced by all five of the isolate FPs. Likewise, every single FP isolate tested positive for several antagonistic characteristics, including antifungal volatile chemicals (HCN). Positive results were also observed for PGP traits, including siderophore synthesis, IAA production, and phosphate solubilization (Table 2). Consequently, VSMKU4040 was chosen to assess the effectiveness of in vivo biocontrol against phyto fungal plant diseases since they were thought to be the most promising antagonistic bacterial strains.

By producing hydrolytic enzymes including chitinase, cellulase, protease, and amylase, the isolates of *Pseudomonas* spp. like AS19 and AS21 were able to considerably reduce phyto fungal plant diseases like *R. solani* f. spp. *sasakii in vitro*. The rhizosphere's beneficial microbes reduce bacterial and fungal diseases through a variety of mechanisms. The primary mechanisms are siderophore and phosphate solubilization, hydrolytic enzyme synthesis, defence enzymes, and hydrogen cyanide (Harikrishnan *et al.*, 2014; Nithya *et al.*, 2019). The rhizospheric FPs of tomato and rice were remarkably productive of a variety of hydrolytic enzymes, up to 20-40%. In contrast, 50% or more of the FP rhizosphere isolates are capable of producing antibiotics and characteristics that aid in plant growth, such as hydrogen cyanide, siderophores, IAA, and phosphate solubilization (Nithya *et al.*, 2020; Suresh *et al.*, 2022b). Previous research indicates that fungal cell wall-degrading enzymes, including cellulase, protease, and chitinase, generate FPs and are primarily in charge of suppressing insects and phytopathogenic microbes.

Identification of *Pseudomonas* spp. VSMKU4040

A variety of biochemical and morphological tests were used to characterize the phenotype of each antagonistic isolate of FPs. While the antagonistic isolates VSMKU4031, VSMKU4036, and VSMKU4028 were negative for VP and MR, all other isolates were motile, rod-shaped, Gram-negative, and positive for catalase, oxidase, and citrate consumption. All of the FP isolates' pyocyanine presence was detected at 365NM using a UV illuminator (Table 3). 1500 bp was the size of the 16s rDNA that was found (Figure 3). Therefore, all things considered, morphology, physiochemical analysis, and the identification of 16 s rDNA in an agarose gel using a specific primer by polymerase reaction demonstrated that the isolate VSMKU4040, which was specifically chosen, was identified as *Pseudomona*s spp. We found that a variety of physiological and biochemical tests, including MR and VP negative, citrate utilization positive, catalase positive, oxidase positive, Gram's reaction negative, and form of all isolates rod, enabled the fluorescent pseudomonades isolates stated by Suresh *et al.,* 2022a, to be discovered to the genus level. Following our findings, we stated that the Gram's reaction, shape, physiological analysis, biochemical analysis, and 16s rDNA encoding gene were used to identify *Pseudomonas* spp. (Figure 3) Furthermore, the primary feature of *Pseudomonas* species is the presence of pyocyanine pigments on King's B agar, which indicates that 100% of the sample belongs to *Pseudomonas* species (Nithya *et al.*, 2019; Varatharaju *et al.*, 2020).

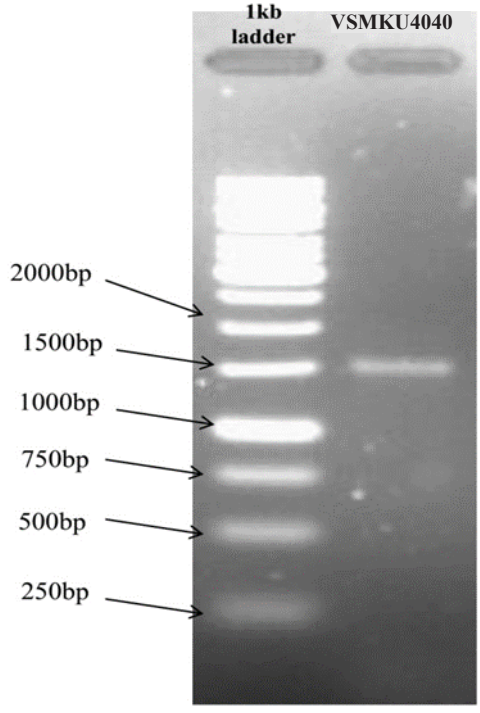


Figure 3. Detection of 16s rDNA encoding gene from *Pseudomonas* spp. VSMKU4040 by Polymerase chain reaction.

Detection of antifungal compounds on TLC

On TLC, three bands in total with Rf values of 0.91, 0.85, and 0.8 were seen. It demonstrated notable suppression against fungal and fungal infections at the Rf value of 0.85 (Figure 4). Therefore, the compound with an Rf value of 0.85 was chosen for further research. Our latest results are in excellent agreement with earlier studies. Following extraction with an appropriate solvent, the microbial antagonistic microorganisms were isolated from a variety of metabolites and were found on TLC in three to four bands. After removing all of the bands from the TLC, the well diffusion method was used to test the bands for antagonistic

Table 3. Phenotypic characterization of FPs

Isolates of FPs	Grams reactions	Fluorescent pigment (Pyocyanine)	Shape	Indole	VP	MR	Citrate utilization	Catalase	Oxidase
VSMKU4031	–	+	Rod	+	–	+	+	+	+
VSMKU 4036	–	+	Rod	+	–	–	+	+	+
VSMKU4038	–	+	Rod	+	+	+	+	+	+
VSMKU4028	–	+	Rod	+	+	–	+	+	+
VSMKU 4040	–	+	Rod	+	+	+	+	+	+

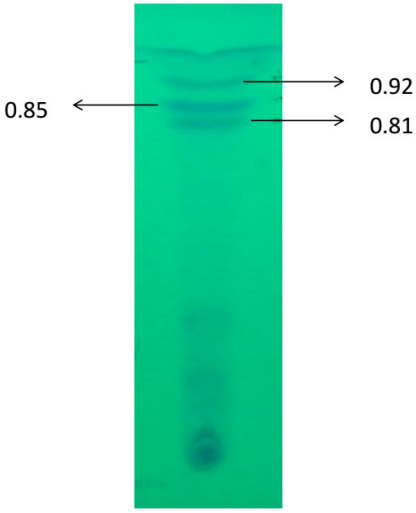


Figure 4. Detection of antifungal metabolites on TLC.

Table 4. Antifungal activity of crude metabolites at 30 µg/ml from *Pseudomona*s spp. VSMKU4040 against fungal phytopathogens under *in vitro* conditions

Fungal phytopathogens	Zone of inhibition(mm)			
	25µl	**50*µl***	**75*µl***	**100µl**
R. solani	6.8±1.5	8.6±0.4	10.8±1.1	27±1
S. rolfsii	9.1±0.7	19±1	21.3±1.5	23±1.5
***F. oxysporum* RACE**	6.3±0.7	6.3±0.7	14±1.5	19±1
F. oxysporum	7.8±0.4	9.1±0.7	17±1	23.6±1.5

activity against fungal phytopathogens such as *R. solani, M. phaseolina, F. ozysporum,* and *Ralstonia solanacearum*. Comparing the outcomes to the control, various degrees of the zone of restriction were detected (Shanmugaiah *et al.*, 2010; Harikrishnan *et al.*, 2014; Nithya *et al.*, 2020; Suresh *et al.*, 2022a). The specific metabolites that exhibit activity against bacterial and plant fungal diseases will be chosen for purification and identification through a variety of spectroscopic and chromatographic analyses.

Antifungal activity of crude metabolite from *Pseudomonas* spp. VSMKU4040 against fungal phytopathogens

Using different concentrations of crude metabolites at 30µg/ml (25, 50, 75, and 100 mg/ml), the antifungal activity of antagonistic *Pseudomonas* spp. VSMKU4040 demonstrated varying levels of ZOI in contrast to control against a variety of phyto fungal plant diseases, including *R. solani, S. rolfsii, F. oxysporum* RACE, and *F. oxysporum*, with varying degrees of inhibition zones ranging from 6.3 mm to 27.0 mm. But when compared to the other fungal phytopathogens and controls, crude metabolites from *Pseudomonas* spp. VSMKU4040 showed impressive antifungal activity against *R. solani* and *S. rolfsii* (Table 4). In par with our findings Suresh *et al.*, (2022a) reported that the control of phyto fungal pathogens by the partially purified diacetyl phloroglucinol (DAPG) produced by *P. fluorescens* VSMKU30504. FPs are widely regarded as effective growth-promoting and biocontrol agents because they produce wide-spectrum bioactive metabolites. More specifically, they have been found to produce very effective antibiotic compounds, such as 2, 4-diacetyl phloroglucinol (DAPG), phenazine-1-carboxylic acid, and phenazine-1-carboxamide (PCA), which leads to antibiosis (Shanmugaiah *et al.*, 2010; Charulatha *et al.*, 2013; Suresh *et al.*, 2022b). Along with standard phenazine and DAPG, some recent findings also showed that crude and purified metabolites from *Pseudomonas* spp. MML2212, *Streptomyces aurantiogriseus* VSMGT1014, *P. aeruginosa* VSMKU1, and *P. fluorescens* VSMKU3054 have significant antagonistic activity against *R. solani, S. rolfsii, F. oxysporum,* and *Macrophomina phaseolina,* with varying degrees of zone of inhibition (Shanmugaiah *et al.*, 2006; Harikrishnan *et al.*, 2016; Nithya *et al.*, 2020; Suresh *et al.*, 2022b). This is also consistent with what we have recently discovered using *Pseudomonas* spp. VSMKU4040 to combat phyto fungal plant pathogens. Additionally, our research shows a strong association between the antagonistic FPs varying degrees of secondary metabolite and hydrolytic enzyme synthesis and the inhibition of fungal phytopathogen growth. This suggests that antibiosis is the primary mechanism of action for the control of phyto fungal and bacterial pathogens and plant disease control.

CONCLUSION

Based on the assessment of hydrolytic enzymes, antimicrobial metabolites, and plant growth hormones, the isolated strain of *Pseudomonas* spp. VSMKU4040 was found to have greater potential to control a variety of fungal phytopathogens. As a result, it may be used as a potent antifungal agent to protect plants from bacterial and fungal diseases. Further research on, *Pseudomonas* spp. VSMKU4040 responsive antifungal metabolites can be purified and characterized for more in depth studies.

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