

Post-harvest protective applications of rhamnolipid producing phyllospheric *Pseudomonas* sp. MP 11 isolated from *Musa acuminata*

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Abstract

Microorganisms residing within the diverse parts of plants play a significant role in the plant growth and defence response. Hence, the current study emphasized the isolation and screening of plant-beneficial traits of isolates obtained from leaves of *Musa acuminata* cultivated in Kottayam, Kerala. Among the many isolates obtained during screening, MP 11 was found to have promising activity against the tested phytopathogens viz. *Fusarium rolfii*, *Pythium aphanidermatum*, *Phytophthora infestans*, *Sclerotium rolfii*. This isolate was further identified as *Pseudomonas* sp. by the 16S rRNA-based sequence analysis. Additionally, the isolate was analysed for various plant growth promoting traits, whereby it showed its capability to solubilise zinc and phosphate, IAA, HCN and ACC deaminase production. MP 11 also showed potential to provide post-harvest protection to the rice seeds from *P. aphanidermatum* and *S. rolfii*. Based on all these properties, the isolated PGPB could be developed into a formulation which would induce multiple beneficial impacts in the production of rice.

Introduction

Post-harvest loss of agricultural products causes significant challenges in the food supply. The storage conditions and the nature of agricultural products are known to favour the growth and multiplication of various fungal agents resulting in the spoilage and even the introduction of mycotoxins. This makes the product unsuitable for human consumption. In 2020, the global percentage of food loss has been estimated as 13.3% (FAO, 2022) and at the same time, mycotoxin contamination has been reported for 25–40% of cereal grains (Kumar and Kalita, 2017). In the case of grains, the post-harvest loss is mainly caused by *Penicillium verrucosum*, *Aspergillus flavus*, *A. ochraceus* and some *Fusarium* species (Magan and Aldred, 2007). In the case of pulses, 6.67% (of total capacity) of post-harvest loss has been reported to be caused by the fungal agents. At the same time, the growth and multiplication of *A. flavus* in ground nut has been reported to contaminate it with carcinogenic aflatoxins (Mesterházy et al. 2020; Norlia et al. 2019). As the agricultural products are directly subjected to the processing steps, the introduction of mycotoxins will have serious impact even at the consumer level. At the same time, various chemicals like hydrogen peroxide (H₂O₂), chlorine-based solutions (NACIO), and peroxyacetic acid (PAA) are used for post-harvest protection (Mahajan et al. 2014). But these chemicals can cause environmental pollution with direct harmful effects to humans which result in life threatening conditions (Artés et al. 2009). Here comes the need for natural or bio-based methods for the post-harvest protection. Based on the recent understanding on plant beneficial microorganisms and their antagonistic mechanisms against spoilage-causing and pathogenic fungal species, it open up new avenues for the exploration of their antifungal chemical bases for the post-harvest applications.

Plant microbiome has recently been studied well for the plant beneficial properties. Based on this, a large number of microorganisms have already been reported to be associated with plants by supporting it beneficially through the production of plant hormones, nutrient mobilisation, and biotic and abiotic stress management (Mendes et al. 2013). Among these, the plant disease protection itself has been reported to be executed by these microorganisms in diverse ways. This includes the plant defence activation through

the physical interactions and also through the antagonism against fungal agents executed through the production of an array of chemical scaffolds and enzymes (Nishad et al. 2020). In the case of plant beneficial bacterial genera like *Bacillus*, *Burkholderia*, *Agrobacterium*, *Serratia*, *Erwinia*, and *Pseudomonas*, a wide variety of antifungal compounds have been reported (An et al. 2022; Bhattacharyya and Jha, 2011). Among the *Pseudomonas* spp., *P. aeruginosa*, *P. fluorescens*, and *P. putida* have been reported to produce the antifungal chemicals like rhamnolipids, 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, pyrrolnitrin, nunamycin, nunapeptin, brasmycin, braspeptin, etc. These compounds are remarkable for the multimechanistically diverse antifungal activity (Sah et al. 2021; Dimkić et al. 2022). In the case of rhamnolipid, *rhlAB* (encoding rhamnosyltransferase 1) and *rhlI* and *rhlR* (involved in regulation) genes are responsible for the rhamnolipid synthesis. The antifungal activity of rhamnolipid is due to the cell wall disruption and rupture of spore membranes of zoospores (Desai and Banat, 1997; Sha et al. 2012). *P. aeruginosa* DS9 has already been reported for the rhamnolipid mediated antifungal activity due to the presence of *rhlAB* gene (Monnier et al. 2018; Olvera et al. 1999; Soberón-Chávez et al. 2021). Also, *Pseudomonas* sp. MP12 isolated from the soil sample has been reported to have inhibitory activity against *Botrytis cinerea*, *Alternaria alternata*, *Aspergillus niger*, *Penicillium expansum*, and *Neofusicoccum parvum* due to the synthesis of antifungal compounds mediated by the expression of antifungal genes like *phlD*, *pltB* and *prnC* coding for DAPG, pyoluteorin and pyrrolnitrin, respectively (Andreolli et al. 2019). Because of these, rhamnolipid producing *Pseudomonas* spp. have significant applications in the post-harvest protection. At the same time, identifying such *Pseudomonas* sp. from specific tissues of plants can enhance its suitability for post-harvest applications.

Plant beneficial microorganisms have already been characterized from diverse parts of plants. Among these, bacteria isolated from the leaves can expect to have highly adapted mechanisms for abiotic and biotic stress management. In a previous study, *Pantoea septica* isolated from the leaves of date palm (*Phoenix dactylifera* L.) has been reported to have the antifungal activity against *Fusarium solani* and *Alternaria* sp. due to the production of antifungal compounds (Al-Nadabi et al. 2021). In another study, endophytic *P. aeruginosa* CP0433 isolated from the leaves of *Anredera cordifolia* CIX1 has been reported to have antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* due to the production of active secondary metabolites with antibacterial and antioxidant activities (Nxumalo et al. 2020). Moreover, endophytic *Bacillus* strains from tomato plants have also demonstrated for antifungal activity against *Botrytis cinerea* (Kefi et al. 2015). Further, *Pseudomonas* P2 strain isolated from the leaf of olive trees was reported for its significant activity against *Rhizoctonia solani* (Elkahoui et al. 2015). Hence the current study has been designed to isolate phyllosphere bacteria from the leaves of *Musa acuminata* due to their wide leaf area which facilitates a wide range of distribution of diverse microorganisms with activity against fungal pathogens (Ceballos et al. 2012). From the results of the study, *Pseudomonas* sp. MP 11 isolated was found to have post-harvest protective effect in rice kernels executed through the production of rhamnolipid.

Methodology

Phytopathogens used

The fungi used in the study were *Fusarium oxysporum*, *Phytophthora infestans*, *Pythium aphanidermatum* and *Sclerotium rolfii* which were collected from the Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India. All the fungi were subcultured weekly and maintained on PDA plates at room temperature.

Isolation of bacteria from the leaves of *Musa acuminata*

In the study, healthy leaves were collected from *Musa acuminata* var. Palayankodan cultivated in Kottayam district of Kerala and used for the isolation of phyllospheric bacteria. For the same, the leaves were washed with distilled water and trimmed into 0.5 x 0.5 cm pieces. Then it was rewashed in sterile distilled water for 3 times. The surface washed pieces of leaves were imprinted on LB and KB agar media and all the inoculated plates were incubated for 2 days at room temperature for the growth of bacteria. Among the colonies formed after the incubation, those with morphologically distinguished features were selected, purified and used for further studies (Savani et al. 2021).

In vitro screening of phyllosphere bacterial isolates for anti-phytopathogenic activity

For determining the antifungal activity of selected bacterial isolates, dual culture and volatile organic compounds (VOCs) based screening were carried out. For the dual culture, bacterial isolates were pre-inoculated on PDA plates for 24 hrs and were further spot inoculated with an agar plug of *F. oxysporum*, *P. infestans*, *P. aphanidermatum* and *S. rolfii* phytopathogens. All the plates were then incubated for 3–5 days at room temperature to evaluate the inhibitory activity against the phytopathogens (Ravi et al. 2021).

Also, VOC-mediated activity against phytopathogens was screened by double Petri dish assay (Calvo et al. 2020). For this, the bacterial culture (100 µL of 1 OD) was spread on the NA plate. This was followed by the inoculation of an agar plug from the edges of actively growing fungal phytopathogens on PDA plates. The two base plates from both sets were sealed with parafilm and incubated at room temperature for 3–5 days. PDA plates with fungal pathogens alone were used as control and triplicates were maintained for each set of experiments. The isolates with promising inhibition against the test fungi from both assays were selected for further studies.

Molecular Identification Of Selected Bacterial Isolate Mp 11 By 16S Rdna Sequencing

For this, the genomic DNA of MP 11 was isolated by using the DNA isolation kit (MN DNA isolation kit, Cat. No. 740952.50). The extracted DNA was further amplified by using 16S rDNA specific primers 16SF (5'-GAGTTTGATCCTGGCTCAG-3') and 16SR (5'-GATATTACCGCGGCGCCTG-3') as per the methods and conditions described in the previous study (Jishma et al. 2021). The PCR product was further sequenced

at the sequencing facility of Agri Genome Labs Pvt. Ltd., Kochi, Kerala, India. The obtained 16S rDNA sequence data was analyzed on EzBioCloud (<https://www.ezbiocloud.net/>) and phylogenetic analysis was done by MEGA11 ver.11.0.10 using the maximum likelihood method with 1000 bootstrap replicates with reference sequences collected from LPSN (List of Prokaryotic Names with Standing in Nomenclature) (Jasim et al. 2013).

Antifungal Activity Analysis Of The Extract From Mp 11

Antifungal activity analysis of the extract from MP 11

Here, the anti-phytopathogenic activity of the MP 11 culture extract was evaluated to confirm the presence of bioactive metabolites with superior antifungal activity. For this, MP 11 was inoculated into 1000 mL of NB and incubated in a shaking incubator at room temperature for 10 days. After this, the culture supernatant was collected and the methanolic extract was prepared. Then, the methanolic extract was further assessed for the inhibitory activity against *P. aphanidermatum* by well diffusion. The experiment was done in triplicates and the tested pathogen alone was maintained as the control (Jasim et al. 2016). The hyphal morphology of the treated and control fungi was also observed in a compound microscope (OLYMPUS BX43F Fluorescence Microscope) at 40X magnification after staining by LPCB method and the images were recorded using Magcam software.

Identification Of Bioactive Vocs By Gc-ms

The VOCs profile of MP 11 was identified by GC-MS analysis using Thermo Scientific (TRACE 1300) GC system. For this, 1 µL of diluted methanolic extract of MP 11 was injected into the capillary column (column size- 30 m x 0.25 mm x 0.25 µm) of GC system maintained with column temperature of 325°C and injection temperature of 300°C Helium was used as a carrier gas at a flow rate of 1.2 ml min⁻¹. The GC oven was held at 70°C and then ramped from 70 to 300°C at 10°C per min. Both chromatogram and mass spectra obtained for the metabolites were further analyzed by comparing the *m/z* peaks with the mass spectrum available in the NIST (National Institute for Standards and Technology) library (Jishma et al. 2017).

Molecular Screening For Antifungal Property

The isolate MP 11 was also screened for the genetic basis of antifungal activity by PCR using the primers specific to the rhamnolipid (*rhIAB*) and pyrrolnitrin (*prnD*) as previously described (Sabu et al. 2019). Amplification of *rhIAB* was carried out using the primers *rhIABf* (5'-CAGGCCGATGAAGGGAAATA-3') and *rhIABr* (5'-AGGACGACGAGGTGGAAATC-3'). At the same time, PRNDF (5'-GGGGCGGGCCGTGGTGTATGGA-3') and PRNDR (5'-YCCGCGSGCCTGYCTGGTCTG-3') primers were used for the *prnD* amplification. The PCR products obtained from the specific amplification reactions were further sequenced and analyzed by EzBioCloud (<https://www.ezbiocloud.net/>) and phylogenetic analysis was also carried out using the

maximum likelihood method with 1000 bootstrap replicates by MEGA11 software ver.11.0.10 (Jishma et al., 2021; Jasim et al. 2016).

Plant Growth Promoting Traits Of Mp 11

The MP 11 was screened for the ability for solubilization of phosphate according to the previously described procedure (Jasim et al. 2013) using Pikovskaya's medium. The inoculated plates were incubated for 24–48 hrs at room temperature and observed for the formation of a clear halo zone around the bacterial colony. The screening for zinc solubilization by MP 11 was done by using the modified procedure described earlier. For this, one loop full (10 μ L) of overnight culture of MP 11 was inoculated onto the basal medium supplemented with 0.2% insoluble zinc oxide followed by incubation for 24–48 hrs at room temperature. The zone of solubilization was measured in mm after the incubation period. Moreover, the IAA production by MP 11 culture was screened by the methods described before (Dinesh et al. 2018). For this, MP 11 at 1 OD was inoculated into 20 mL NB augmented with 0.2% (v/v) of L-tryptophan followed by the incubation at room temperature for 10 days. Afterwards, the culture was centrifuged at 1008 g for 20 min at 4°C and the supernatant was collected. Then 1 mL of the supernatant was transferred into a sterile tube and mixed with 2 mL of Salkowski's reagent (50 mL 35% of HClO_4 and 1 mL 0.5 M FeCl_3 solution) followed by incubation for 30 min at room temperature in dark. The formation of a red colour was checked as indication of IAA production. The ACC deaminase production by MP 11 was also screened by inoculating it on DF salt minimal medium containing 2 g/L ammonium sulphate. The plate was incubated at room temperature for 24–48 hrs and the presence of growth was checked. Besides, the qualitative analysis for HCN production by MP 11 was carried out using the modified method explained before (Kloepper et al. 1991). For this, 5 mL of NB was supplemented with glycine (4.4 g/L) and was inoculated with 1 OD adjusted culture of MP 11. Into this, sterilized filter paper strips soaked in picric acid solution (2.5 g picric acid and 12.5 g Na_2CO_3 in 1 L distilled H_2O) were inserted. The tubes were sealed with parafilm and incubated at room temperature for 24–48 hrs and observed for the change in the colour.

Protective effect of MP 11 on *Oryza sativa* against *P. aphanidermatum* and *S. rolfsii*

The protective effect of MP 11 on *O. sativa* against *P. aphanidermatum* and *S. rolfsii* was studied by using the modified inoculation method (Jasim et al. 2014). For this, the seeds of *O. sativa* purchased from the local paddy field were washed with tap water followed by treatment with 1% sodium hypochlorite for 10 min. This was further followed by wash with sterile distilled water for 3–4 times. For the treatment, three sets of rice seeds were used. The first set was dipped in MP 11 supernatant for 2 hrs, the second and third sets were dipped in NB and distilled water for 2 hrs respectively as controls. The supernatant of MP 11 was prepared by collecting it from 10 days old culture by centrifugation at 12298 g at 4°C. All the treated sets were transferred into sterile Petri dishes for drying for 2 hrs and then inoculated with agar plugs of *P. aphanidermatum* and *S. rolfsii* separately. All the six groups were incubated for 14 days at room temperature and observed the changes on a daily basis.

Results

Isolation Of Bacteria

In the study, 26 bacteria could be isolated from the phyllosphere of *Musa acuminata* which included, 9 from LB and 17 on KB agar media used for the isolation.

Antifungal Activity Of Isolated Bacteria Against Phytopathogens

All the bacterial isolates were screened for the antifungal activity against *F. oxysporum*, *P. aphanidermatum*, *P. infestans* and *S. rolfii*, and among these MP 11 was found to have the highest activity (S. Figure 1). The mycelial radial growth analysis of phytopathogens indicated the highest antagonistic activity of MP 11 to be against *F. oxysporum*.

Voc Mediated Activity Against Phytopathogens

The overlapping plate assay result showed the ability of MP 11 to control the growth of fungal pathogens. Here, MP 11 was found to significantly inhibit the mycelial growth of *P. infestans* when compared with the control. However, the growth of *F. oxysporum* was found to get not significantly affected by the VOC activity of MP 11 (Fig. 1).

Molecular Identification Of Mp 11

The BLAST analysis of the 16S rDNA sequence of MP 11 showed its highest similarity of 100% to *Pseudomonas* sp. strain NBRC 12689 (NR_113599.1). Phylogenetic analysis of 16S rDNA of MP 11 further confirmed its clustering with reference sequences collected from LPSN. Here, the MP 11 sequence was found to cluster perfectly with the clade of *Pseudomonas* spp. (S. Figure 3). *P. aeruginosa* is the other species related to MP 11 as sister clades sharing a common ancestor.

Antifungal Activity Of Methanolic Extract Of Mp 11

The methanolic extract obtained from MP 11 also showed prominent antifungal activity against *P. aphanidermatum* by well diffusion when compared with other phytopathogens (Fig. 2).

Identification Of Volatile Metabolites Produced By Mp 11 By Gc-ms Analysis

The volatile metabolites produced by MP 11 were identified by GC-MS analysis. The major compound identified was Hexadecanoic acid, methyl ester with retention time (RT) of 19.784.

Amplification of *rhlAB* and *pqqC* genes from MP 11

Plant beneficial genes of MP 11 like the *rhlAB* and *pqqC* genes were amplified by PCR and has resulted in the formation of products with 777 and 546 bp size respectively (Fig. 3). The BLAST analysis of PCR products further showed its similarity (%) to WP_023121968.1 and MXH35294.1 respectively. Phylogenetic analysis of *rhlAB* and *pqqC* gene sequences 1,3-(1-hydroxydecanoyloxy)decanoate synthase of *P. aeruginosa* and pyrroloquinoline-quinone synthase PqqC of *P. aeruginosa* along with reference sequences from NCBI also showed distinct clustering (S. Figure 3).

Identification Of Rhamnolipid Production By Mp 11 By Lc-ms Analysis

The LC-MS analysis of the methanolic extract of MP 11 confirmed the presence of mono-rhamnolipid ($C_{26}H_{48}O_9$) with a base peak of m/z 503.1796 ($M + H^+$).

Plant Growth Promotion Analysis

The isolate MP 11 was screened for the phosphate and zinc solubilisation properties and here it showed a clear halo zone around the inoculated area which indicated the ability of the isolate for phosphate solubilisation. Further IAA production screening also showed the formation of reddish pink colour due to bacterial growth and ACC deaminase activity of MP 11 also indicated by its growth on the minimal medium. The isolate also showed the ability for HCN production due to the change in colour of the filter paper soaked in picric acid solution (S. Figure 6).

Protective effect of MP 11 on *O. sativa*

In the *in vivo* disease protective effect screening of MP 11 on *O. sativa*, the MP 11 treatment was found to provide significant protection to the plant against *P. aphanidermatum* and *S. rolfsii*. Here, the protection of rice seeds against *P. aphanidermatum* was found to be significantly greater with almost complete protection when compared to the protection from *S. rolfsii* (Fig. 5).

Discussion

Post-harvest challenges cause substantial loss of agricultural products during its handling, transportation and storage. To control the same, various synthetic chemical agents are used which ultimately result in serious health and environmental problems. Hence the application of efficient biological methods is essential to minimise the use of hazardous synthetic agents for post-harvest applications. Several microbial biocontrol agents have the potential to be applied against the pathogenic and spoilage causing microorganisms for post-harvest protection (Droby et al. 2022).

Microbial infestation of fruits and vegetables during the harvesting is a serious issue which will be intensified during its transportation and storage. Here comes the relevance of biocontrol agents which

can provide resistance to agricultural products by acting as a barrier to pathogenic microorganisms through diverse mechanisms. Such protective effects of food items can significantly ensure the quality of the food materials used further for consumption (Carmona-Hernandez et al. 2019). Hence, the identification of beneficial microorganisms from novel source is essential to explore its application for post-harvest protection. Even the cell free supernatant of such beneficial organisms can have the potential to be applied as coating material for post-harvest applications.

The present study has focused on the isolation, identification, characterization and analysis of microorganisms from the leaves of *Musa acuminata*. The leaves of *M. acuminata* have already been reported for the anti-inflammatory, antioxidant and anti-diabetic properties (Sonibare et al. 2018). The ethanolic extract of *M. acuminata* leaves has also been reported to have inhibitory activity against methicillin-resistant and susceptible *Staphylococcus aureus* (Sivasamugham et al. 2021). Due to the waxy coating and the hydrophobic nature of leaf surface of *M. acuminata* and also because of its continuous interaction with fungi and bacteria, it can be well expected to have the presence of beneficial microorganisms with biocontrol properties. Various species of *Bacillus*, *Pseudomonas* and *Serratia* are well characterised for their antifungal mechanisms executed through diverse metabolites and enzymes (Thomashow & Weller, 1996; Sansinenea, 2019). The *Pseudomonas* spp. have wide environmental distribution which indicate its diverse metabolic adaptations and are generally considered as promoters of plant elongation with significant impact on plant disease protection. The rhamnolipids produced by *Pseudomonas* spp. are also known to act as microbe associated molecular pattern which could activate the plant defense by binding to pattern recognition receptors (PRR). In this study, out of 26 bacterial isolates screened, *Pseudomonas* sp. MP 11 was found to have promising antifungal activity against *F. oxysporum*, *P. aphanidermatum*, *P. infestans*, and *S. rolfsii* in both dual culture and VOC mediated assays which might be due to the broad range of antagonistic mechanisms present in MP 11 against plant pathogens. In a recent study, *P. fluorescens* has been reported to have the antifungal activity against the *Macrophomina phaseolina* which could infect soybeans (Castaldi et al. 2021). In another study, *P. fluorescens* BNM296 isolated from the rhizosphere has been reported to have antifungal activity against *Pythium ultimum* (León et al. 2009). Besides, *P. chlororaphis* subsp. *aureofaciens* SPS-41 has been reported to have antifungal activity against *Ceratocystis fimbriata* through the release of VOCs which contained the antifungals like 3-methyl-1-butanol, phenylethyl alcohol, and 2-methyl-1-butanol (Zhang et al. 2021). Furthermore, *P. putida* BP25 isolated from the black pepper has been demonstrated to have VOCs mediated inhibition of pathogens like *P. capsici*, *P. myriotylum*, *R. solani*, *C. gloeosporioides*, *A. rolfsii*, *G. moniliiformis*, *M. oryzae*, and *R. pseudosolanacearum*. These results indicate that the *Pseudomonas* spp. to have broad range antagonistic principles against plant pathogens and have already been characterised from diverse parts of plants. In the current study, the MP 11 was also found to have the presence of *rhlAB* gene coding for antifungal rhamnolipids. As the rhamnolipids are already known to have activity against diverse plant pathogens, the bacteria selected in the study can consider to have the promises for biocontrol applications. In a previous study, rhizospheric *Pseudomonas* sp. isolated from *Cyclea peltate* was reported to have antifungal activity against *P. myriotylum* due to the production of rhamnolipids (Jishma et al. 2021). In the study, the MP 11 was observed to have the

produced rhamnolipid as identified by LC-MS analysis where the compound with $M + H^+$ (503.17) correspond to rhamnolipid. By combining the results of PCR and sequencing of *rhIAB* gene of MP 11 along with the LC-MS result, the isolate can consider to have rhamnolipid as a key compound to be responsible for its antagonism against selected plant pathogens. In a previous study, mechanistic basis of antifungal activity of rhamnolipid has been demonstrated to be due to the direct interaction with membrane lipids and thereby affecting the integrity of plasma membrane. This could further induce the targeted cell death of the pathogenic microorganisms very quickly (Botcazon et al. 2022; Crouzet et al. 2020). The isolate MP 11 has also been described for the volatile mediated antifungal activity. This could be due to its HCN production also with the activity of blend of VOCs like propane-1,2-dimethoxy-, methyl pyruvate dimethyl acetal, and 9-decenoic acid. as identified by GC-MS analysis. In a previous study, Hexadecanoic acid, methyl ester has already been described for the antimicrobial activity as observed in the current study (Shaaban et al., 2021). The demonstrated antifungal properties of MP 11 is clear indication of its application for post-harvest protection which is an emerging area of application of plant beneficial microorganisms.

The isolate MP 11 was also found to be positive for various plant growth promoting properties like phosphate and zinc solubilisation, IAA production, and ACC deaminase activity. The ability of transforming insoluble to soluble phosphate is one of the important characteristics for improving the crop productivity as the phosphorus is considered to be a limiting factor for plants (Sah et al. 2021). Zn deficiency is a major problem in the agriculture field and it could severely affect the growth, development, maturity and productivity of plants. Hence the identification and application of zinc solubilising microorganisms has already been considered to have a huge impact on the plant growth and yield. Various bacterial genera including, *Bacillus* and *Pseudomonas* have already been reported to solubilise insoluble zinc to soluble forms extensively (Hashemnejad et al. 2021). Moreover, the capability for IAA production could be beneficial for the host plant for better uptake of nutrients and thereby the overall plant growth. It could have determining effects on the cell enlargement, cell division, root initiation, and apical dominance in plants (Ahemad & Khan, 2012). Many *Pseudomonas* spp. have previously been reported to express the ACC deaminase production property which hence could confer the tolerance to plants against stress factors by lowering the ethylene level (Win et al. 2018). As the bacteria isolated from this study, *Pseudomonas* sp. MP 11 has also been described for plant beneficial traits, it can also have the promising application in agricultural fields to promote plant growth and productivity.

The isolate MP 11 has also found to have protective effect on rice kernels infected with *P. aphanidermatum* and *S. rolfisii*. *Pseudomonas* strains isolated from the rhizosphere have already been described for *in vitro* antibiosis against *Xanthomonas oryzae* and *R. solani* under normal and saline conditions (Rangarajan et al. 2003). In another study, *P. fluorescens* PfALR2 was found to have inhibitory activity against *R. solani* under field trial when applied as a pet-based formulation (Rabindran & Vidhyasekaran, 1996). Moreover, *Pseudomonas* spp. have been reported for their post-harvest protective effect against various fungal species. For instance, *Pseudomonas* sp. isolated from the rhizospheric soil have described for the potential inhibitory activity against *Penicillium digitatum* infecting citrus fruit

during the post-harvest (Qessaoui et al. 2022). Also, rhizospheric *P. aeruginosa* BRp3 has demonstrated for biocontrol activity against *X. oryzae* in Basmati rice due to the production of secondary metabolites including rhamnolipids (Yasmin et al. 2017). Hence the protective activity exhibited by MP 11 supernatant against *P. aphanidermatum* and *S. rolfsii* in rice seeds might be due to the presence and activity of antifungal metabolites especially rhamnolipids.

The present study also focused on the isolation and identification of potential antifungal metabolites from MP 11. Because, *Pseudomonas* sp. MP 11 was found to have significant inhibitory activity against phytopathogens. Here, the GC-MS and LC-MS analyses revealed the presence of major antifungal metabolites including rhamnolipids. The amplification of genes *rhIAB* and *pqqC* further confirmed the ability of MP 11 to produce metabolites with antifungal mechanisms and plant growth promotion potential. Eventhough various *Pseudomonas* spp. have already been isolated from various resources, the isolation of MP 11 is the first report on the isolation of *Pseudomonas* sp. from *M. acuminata* which highlights the importance of the study. Also, the post-harvest protective effect of *Pseudomonas* sp. against *P. aphanidermatum* and *S. rolfsii* is the first report in this field. Therefore, *Pseudomonas* sp. MP 11 isolated in the study can have the promises to develop as a microbial formulation to apply on various economically important crops, during post-harvest stage targeting different fungal pathogens.

Declarations

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Contributions

Maria Liz George, Amala Geevarghese and Athira Anilkumar involved in the methodology and data curation. Mary Theresa involved in the data curation, original draft preparation and editing.

Radhakrishnan E K involved in the supervision, conceptualization, validation, reviewing and editing. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest regarding this research.

Ethical approval

This study does not contain any experiments with human participants or animals.

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Figures

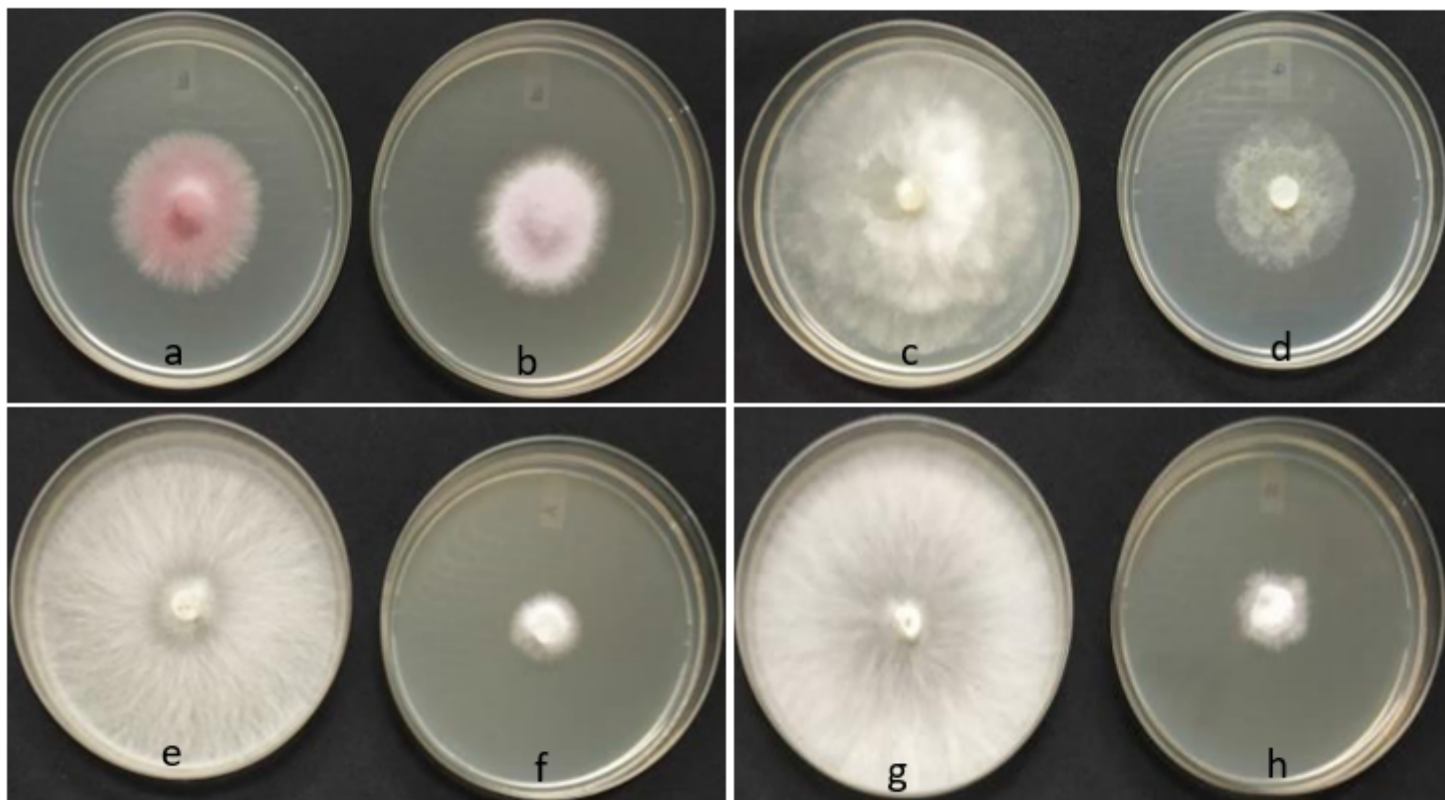


Figure 1

VOCs mediated antifungal activity of MP 11. (a) *F. oxysporum* control, (b) activity of MP 11 against *F. oxysporum* (c) *P. aphanidermatum* control, (d) activity of MP 11 against *P. aphanidermatum* (e) *P. infestans* control, (f) activity of MP 11 against *P. infestans*, (g) *S. rolfsii* control and (h) activity of MP 11 against *S. rolfsii*.

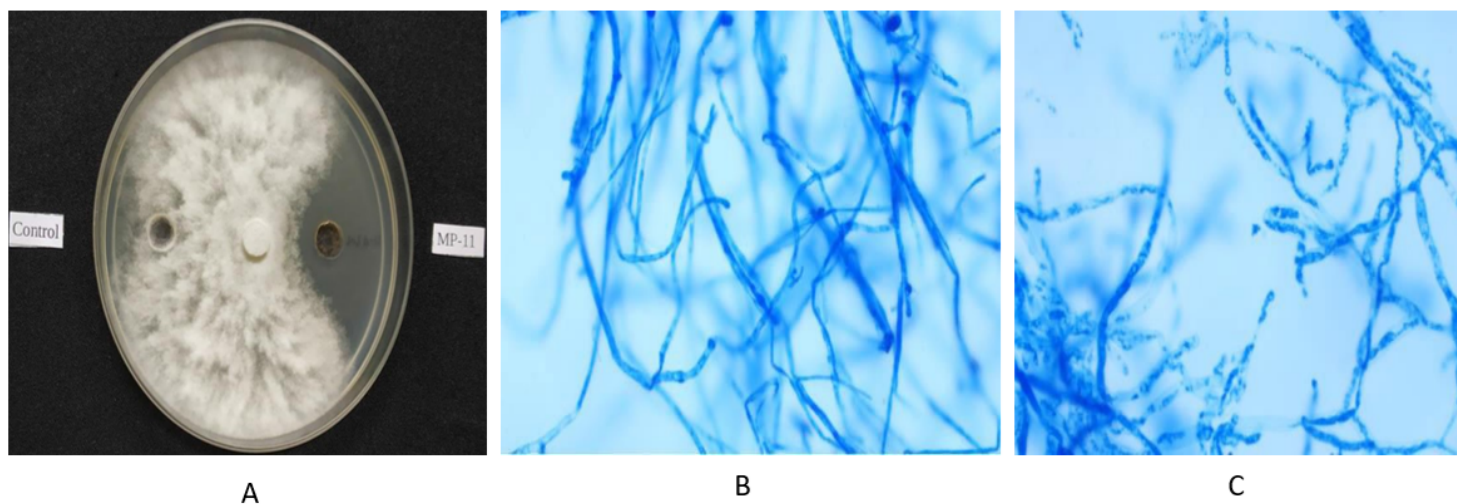


Figure 2

Antifungal activity of MP 11 extract. A- Activity of the extract of MP 11 against *P. aphanidermatum*. Microscopic observation on antifungal activity of the extract of MP 11 with (B) Hyphae from the control plate and, (C) Fungal hyphal damage due to the activity of MP 11.

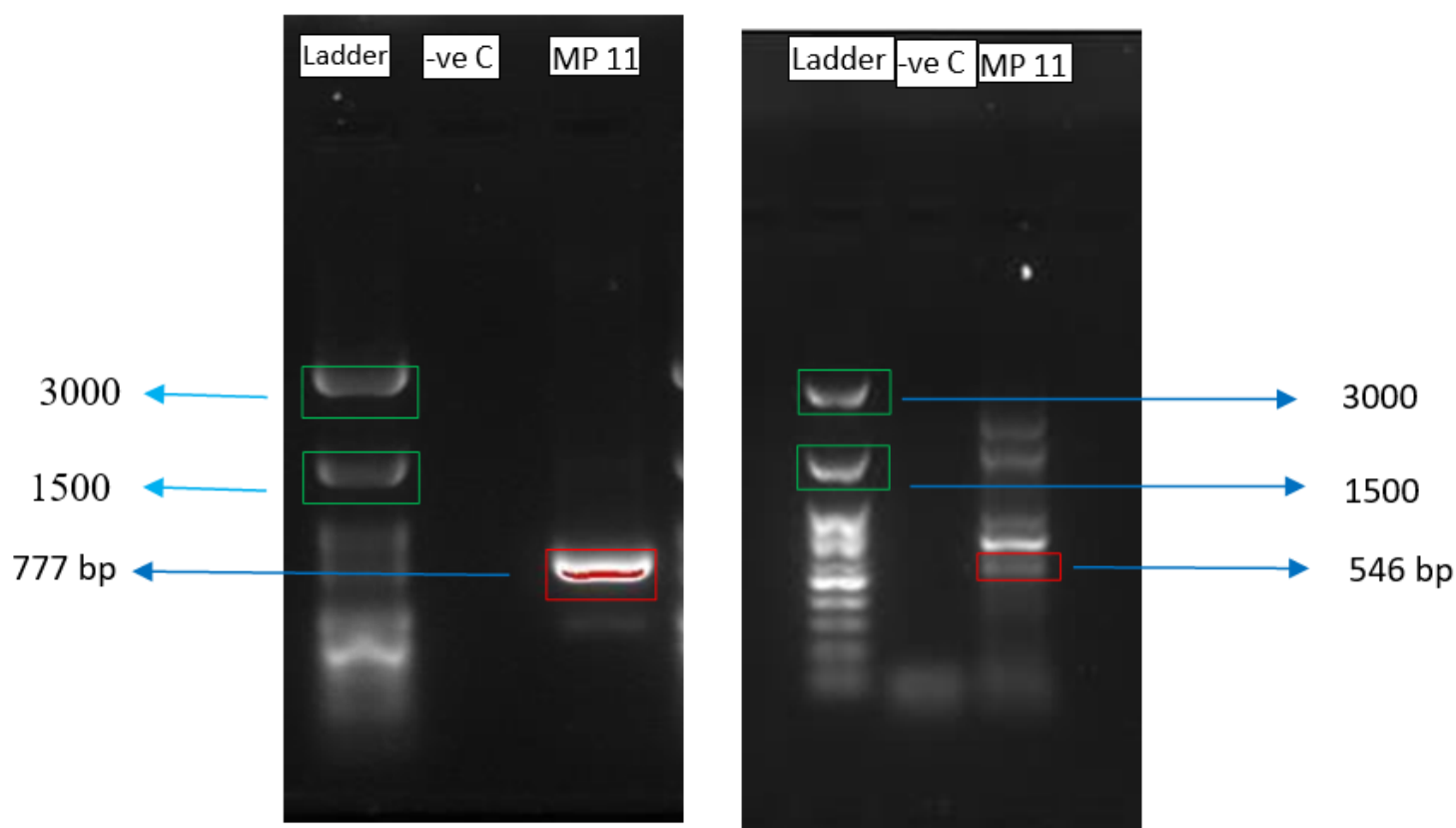


Figure 3

PCR amplification of plant beneficial genes (A) *rhlAB* and (B) *pqqC* from MP 11 showing bands at 777 and 546 bp respectively.

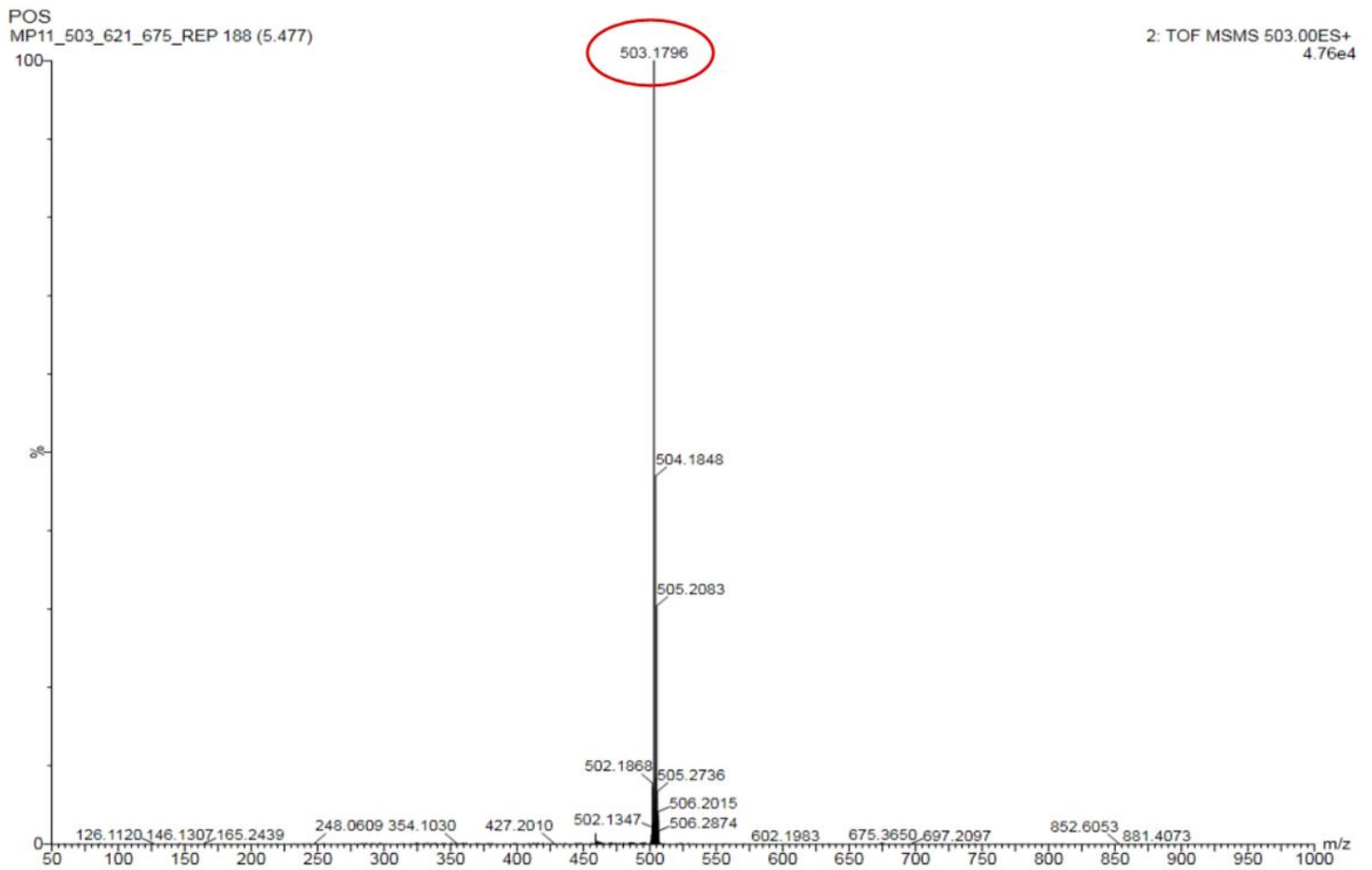


Figure 4

LC-MS analysis of MP 11 indicating the presence of rhamnolipid with m/z of 503.1796 ($M + H^+$).

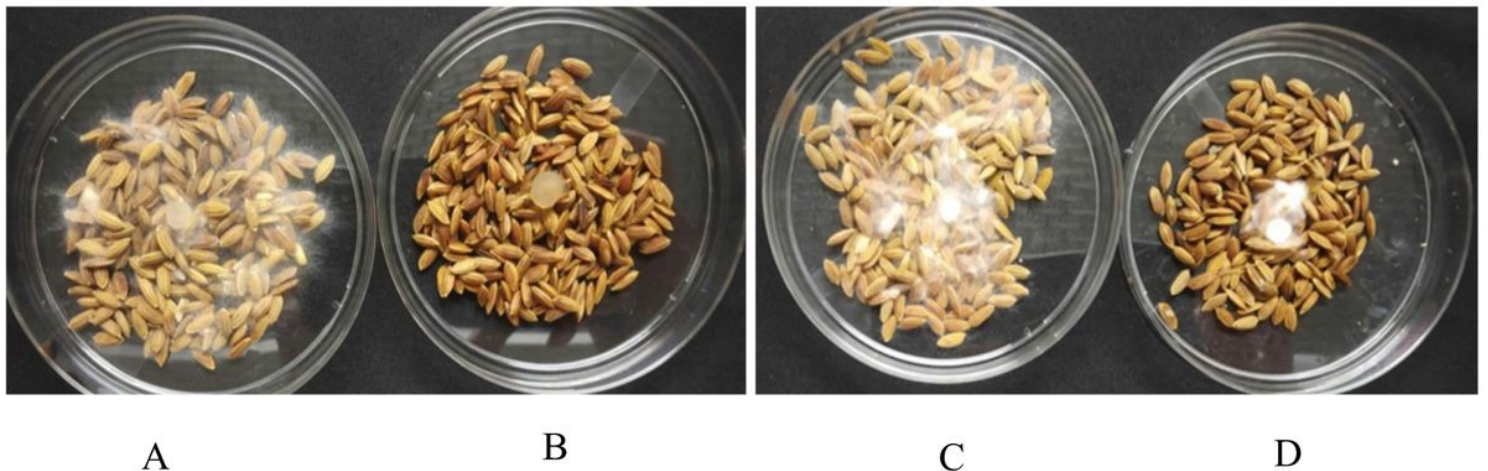


Figure 5

*In vivopost-harvest protection of rice against *P. aphanidermatum* and *S. rolfsii* by MP 11. A- *P. aphanidermatum* in control rice soaked in NB medium, B- Protection of rice by MP 11 against *P.**

aphanidermatum, C- *S. rolfsii* infection in control rice soaked in NB medium, D- Protection by MP 11 against *S. rolfsii*.

Supplementary Files

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