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Enhanced siderophore production by *Pseudomonas aeruginosa* and its antagonism against fungal threats in sesame fields

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Abstract

We succeeded in identifying and isolating three strains of *Pseudomonas aeruginosa,* namely, P2LA3, N3D3 and KMND3, from the soil of sesame (*Sesamum indicum* L.) cultivation fields in Onattukara, Alappuzha district, Kerala, India. Further in-depth microbiological studies reveal that the maximum siderophore yield is observed in strain KMND3 (70.9 µM), followed by P2LA3 (54 µM) and N3D3 (27 µM). All these strains showed considerable antagonism against significant sesame fungal pathogens such as *Aspergillus flavus, Fusarium moniliforme, F. oxysporum and Rhizoctonia solani*. Among these strains, KMND3 proved to be the most effective. Therefore, we focused on this strain for further study to demonstrate the influence of various physico-chemical parameters on siderophore production. This study identified several parameters that enhance production, such as starch as a carbon source, yeast as a nitrogen source and supplementary media containing Cd^{2+}, Mn^{2+} and Hg^{2+}. In contrast, a high concentration of iron was found to inhibit production. The results of our study confidently highlight the potential of *P. aeruginosa* strain KMND3 as a successful bio-control agent capable of suppressing fungal pathogens in sesame through siderophore-based mechanisms.

Keywords

antagonism; biocontrol; Kerala; microbiology; PGPR; sustainable agriculture

Introduction

Sesamum indicum L. (Pedaliaceae), known as Indian sesame, is an essential oil-yielding species worldwide. Sesame seeds are a rich source of unsaturated fatty acids (1). India is the second largest sesame producer, but its national output has decreased by 1.8 % annually since 2014 (2, 3). In Kerala, sesame production is very low and its cultivation is restricted to the Onattukara region of the Alappuzha district. Although it is specified, the output from this region is well known and received a Geographical Indication Tag on 30 Nov. 2022 because of its unique colour and high levels of vitamin E and antioxidants (4). There are reports on the utilization of Onattukara Sesame by local Ayurvedic practitioners since the 18th century to treat rheumatism and skin diseases. The sesame plant is Indias' "seed of mortality" and has several therapeutic uses. Lignan found in sesame seeds are crucial in treating atherosclerosis and the ageing process by lowering the bodys' cholesterol levels. Alpha-tocopherol can decrease the incidence of cardiovascular illnesses and several types of cancer. The fatty acids in seed oil have antirheumatic, antithrombotic, hypolipidemic and vasodilatory qualities. It is used as a mouthwash in India to alleviate headaches, dizziness, impaired vision, anxiety and insomnia (5). Crop production in Onattukara mainly depends upon different environmental and seasonal variations, including stress due to heavy rainfall, floods and diseases. Various diseases

affecting sesame are thought to cause the loss of 7 MMT annually (6). diseases caused by *Fusarium oxysporum* f. sp. sesami, *Alteraria sesami, Colletotrichum gloeosporioides*, *Curvularia lunata* and *Botryodiplodia* significantly decreased the yield, according to loss estimation tests and A*spergillus flavus*, A. *niger*, R*hizopus nigricans* and M*ucor haemali* were the prevalent fungi linked to sesamum seeds in Kerala (7). Farmers in this area mainly depend on organic fertilizers and pesticides and are aware of hazards associated with chemical usage.

Most crops, including sesame, are infected by *Aspergillus flavus,* a common soil fungus producing aflatoxin (8). Another pathogen, *Fusarium oxysporum,* which is typically harmless and part of the rhizosphere microbial population, causes *Fusarium* wilt (9). *F. oxysporum* and *F. sesami*, which cause seedling blight and wilt diseases and survive inadequate fungicidal treatment, result in seasonal fluctuations in crop output that fall short of meeting the countrys' needs. *Fusarium moniliforme* is another potential sesame pathogen responsible for blight on seedlings and root rot disease (10).

In recent years, special attention has been paid to various rhizobacteria as bio-control agents against these pathogens (11). These bacteria support plants through multiple processes, including the production of phytohormones, siderophores, antioxidants, cell wall-degrading enzymes that break down fungal cell walls and volatile organic compounds (VOCs). Furthermore, there are direct benefits from biological nitrogen fixation and the solubilization of minerals, including zinc, potassium and phosphorus. According to (12), under iron stress, most fluorescent Pseudomonas may employ bacterial iron siderophore complexes as their primary source of iron from the soil, giving them a competitive edge that prevents phytopathogens from growing and colonizing their roots. This implies that a key biological control mechanism for several luminous pseudomonads is siderophore-mediated competition for iron with pathogenic soil microbes. Antibiotic influence on suppressing pathogens through colonization, resource competition, parasitism and mycophagy are potential pathways for antagonistic activity (13). In the present study, soil from fields in Onattukara was screened to identify bacterial species with *in vitro* efficiency against phytopathogenic fungi.

This work aimed to identify and optimize the siderophores generated by the *Pseudomonas aeruginosa* strains and explore its potential as a bio-control agent against fungal species that cause mortality in sesame. In the optimization experiment, we examined how the strains' ability to make siderophores was impacted by pH, Fe content, carbon source, nitrogen supply and heavy metals. *Pseudomonas aeruginosa* also demonstrated Plant growth promoting rhizobacteria characteristics and as a biocontrol agent, it provided a practical mechanism for defeating phytopathogens (14). In addition, its potential for siderophore-assisted bioremediation of hazardous pollutants is relevant (15). Commercializing these bacteria can achieve sustainable agricultural goals and aid in managing various fungal plant diseases by preventing iron-scavenging pathogens by applying siderophore-producing bacteria as bio-inoculants.

Materials and Methods

Study area

The bacterial isolation was conducted by collecting soil samples from the rhizosphere of Sesame (*Sesamum indicum* L.) fields in the Onattukara region of Alappuzha district in Kerala, India. The sandy plains that stretch from the coast into the midlands and encompass the Kollam District and Alappuzha District are included in the unique agro-ecological unit known as the Onattukara Sandy Plain. The sandy, deep, well-drained soils have a low water table. The acidity of the subsoil is rising. Medium-sized soil organic matter is limited to retaining water and nutrients and experiences 22 °C to 25 °C temperatures and coastal climate.

Isolation of Pseudomonas aeruginosa from the soil of the study area

Rhizosphere soil samples of 100g each were collected from the random spots in the study area. From those, 1ml of bacterial suspension was prepared and considered for the serial dilution from 10^{-1} to 10^{-8}. After that, the diluted suspension was inoculated in Kings B (KB) agar medium (KB: D.W. 1000 ml; Peptone 20 g; Glycerol 10 g; $MgSo_4$ 1.5 g; K_2PO_4 1.5 g). Kings Medium B Base is well-suited for detecting and enumerating fluorescing bacteria and synthesizing pyocyanin and pyorubin.

Genomic sequencing and identification of the strains

The bacterial samples from the inoculation were taken to isolate genomic DNA for sequencing. This isolation was performed using the NucleoSpin® Tissue Kit (Macherey-Nagel) and agarose gel electrophoresis was used to assess the integrity of the extracted DNA. Using a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems), 0.25 µL of forward primer "CAGGCCTAACACATGCAAGTC" and 0.25 µL of reverse primer "GGGCGGWGTGTACAAGGC" together with 5µL2X Phire Master Mix, 4µL distilled water and 1µL of DNA, the PCR amplification was performed.5µL of PCR product was combined with 0.5µl of ExoSAP-IT and the mixture was incubated at 37oC for 15 minutes. The enzyme was then inactivated at 85 °C for 5 minutes to eliminate any remaining primers and dNTPs. The BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) performed the sequencing reaction in a PCR thermal cycler (GeneAmp PCR System 9700). The settings for the response were as follows: an early denaturation stage at 95 C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 60 °C for 40 seconds, followed by 72 °C for one minute and the final extension procedure at 72 °C for a total of 7 minutes. 50 µl of a mixture consisting of 5µL D/W, 0.1 µl 125mM EDTA, 1 µl 3M sodium acetate pH 4.6 and 44 µl ethanol was used to cleanse the PCR products. The Sanger genome sequencing method was used to sequence the cleaned and air-dried product in an Applied Biosystems ABI 3500 DNA Scanner. All these DNA isolation and sequencing processes were carried out at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Tests of bio-control determinants

Siderophore spectrum analysis and estimation

One hundred ml of a 24-hour-old bacterial culture (1×10^8 colony forming units (cfu) per ml) was injected into 100 ml of iron-deficient succinate media and incubated in a rotatory shaker set at 30 ℃ for 24 hrs at 120 rpm (16). The culture was centrifuged at 10000 rpm for 10 minutes and the absorbance of the supernatant was measured at 400 nm to determine the amount of siderophore produced. The quantity of siderophore was calculated using the molar extinction coefficient (⊠ = 20,000/M/cm) and the absorption maxima (λ = 400 nm) (17).

Fungal strains for antibiosis assay

Four fungal pathogens commonly reported in sesame plants, namely, *Fusarium moniliforme* (MTCC6985), *Aspergillus flavus* (MTCC183), *Fusarium oxysporum* (MTCC 284), *Rhizoctonia solani* (MTCC 4634) were procured from IMTECH (CSIR), Chandigarh, India.

In-vitro antibiosis study

The antagonistic capability of *Pseudomonas* was evaluated using streak inoculation. A 24-hour-old bacterial culture was streaked in four curves, 2 cm away from the Petri plates' periphery, containing Kings' B medium agar. It was kept incubated for 48 hrs at 36°C ± 2°C. Then, a fungal disk of the selected pathogen (4 mm) was placed on the centre of the petri plate. It was further incubated in a BOD incubator at 36°C ± 2°C. From the 3rd day of incubation, the inhibition zone is measured in mm. At 24-hour intervals, the inhibition zone is measured up to 5th day. Control plates of Kings' B medium agar contained only fungal discs (18)

Optimization of physio-chemical parameters for siderophore production

For improved siderophore synthesis in the bacterial strain KMND3, parameters such as various carbon and nitrogen sources, pH levels and iron and heavy metal concentrations were tested in a succinate medium.

Effect of pH

It was examined how siderophore synthesis was affected by pH variations (3,5 and 7, with a 2-step interval). After being individually inoculated with the 100µL log-phase bacterial culture (1×10^8 cfu/mL),100 mL of succinate broth was inoculated with 100 µL of log-phase bacterial culture (1×10^8 CFU/mL) and incubated at 120 rpm and 37°C for 72 hours. One ml of each succinate medium and cell filtrate was mixed and an absorption peak was measured between 200 and 600 nm.

Effect of Iron concentration

To identify the threshold level that inhibited siderophore formation.100 µL of log-phase bacterial culture (1×10^8 CFU/mL) was inoculated in succinate broth with $FeCl_3$ $6H_2O$ at concentrations of 25, 50 and 100 µM and incubated in a rotary shaker at 120 rpm for 24-48 hours at 37°C One ml each of succinate medium and cell filtrate was mixed to measure the absorption peak (19).

Effect of carbon and nitrogen sources

100 µL of log-phase bacterial culture (1×10^8 CFU/mL) was inoculated in 100 mL of succinate broth supplemented with four carbon sources (1 g/L each), including fructose, starch and sucrose. It was then incubated in a rotatory shaker for 24-48 hours at 120 rpm at 37°C. 1mL of each succinate medium and culture filtrate (1:1) was mixed after 48 hours of incubation. The absorbance was determined between 200-700 nm (20).

100 µL log-phase bacterial culture (1×10^8cfu/mL) inoculated in succinate broth (100 mL) that included independent supplements of 1g/L of nitrogen sources such as yeast extract, protease Peptone and Potassium nitrate. 1 ml of succinate medium (1:1) was combined with 1 ml of the culture filtrate after 48 hours. The absorbance was determined between 200-700nm (21).

Effects of heavy metals

To determine how heavy metals affect the production of siderophores, 10 µM of three distinct heavy metals-manganese ($MnCl_2$), mercury ($HgCl_2$) and cadmium ($CdCl_2$)-were added individually to each 100 mL of succinate broth. After being incubated for 24-48 hours at 30°C, 1 mL of culture filtrate was mixed with 1 mL of succinate medium and the siderophore content was calculated using the UV-visible absorption spectrum.

Statistical analysis

Data were analyzed using SPSS statistical software. Two-way ANOVA and Duncans' Multiple Range Test (DMRT) were applied for antagonism studies. In siderophore estimation, One-way analysis of variance was used for the statistical analyses and the mean ± standard error (SE) was the result. The tests' statistical significance was assessed at the reliability level of $p < 0.05$.

Results and Discussion

Identified Bacterial strain

Gene sequences of bacterial strains were identified using the blast search tool. These sequences were submitted to GenBank, where they were assigned the accession numbers P2LA3 (ON329827), N3D3 (ON329826) and KMND3 (ON329825). Bacterial strains were cultured in KB medium (Fig. 1).

Siderophore production and its quantification

All three bacterial isolates-KMND3, P2LA3 and N3D3-produced significant amounts of siderophores (Fig. 2). The production of siderophores has been connected to the ability of pseudomonads to reduce diseases (22). The contrasting effects of siderophores and other non-siderophore metabolites on soilborne *Ralstonia solanacearum*, a bacterial pathogen in the tomato rhizosphere, were observed. In contrast, the siderophore effects were relatively much more substantial. Specifically, disease incidence was reduced *in vivo* when the inoculated consortia produced siderophores that the pathogen could not use for its growth (23). These microorganisms invade the roots of various crops, including vegetables, cereals, pulses and oilseeds (24). KMND3 produced the highest concentration of siderophores (70.9

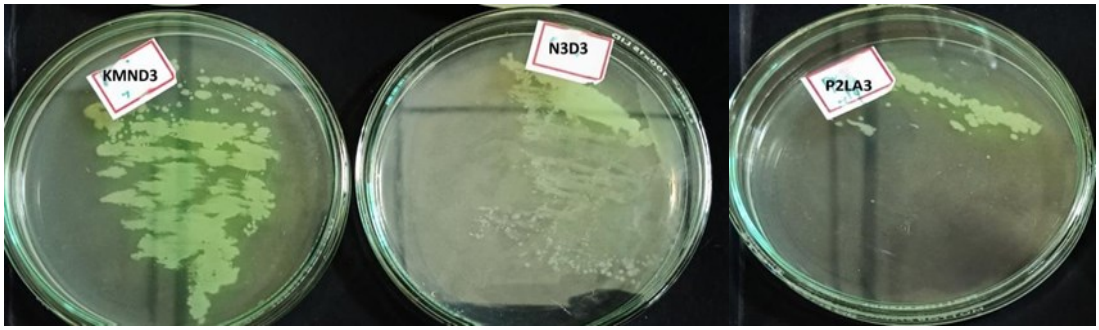

Fig. 1. Isolated strains of *P. aeruginosa* KMND3, N3D3 and P2LA3 plated on KB agar medium.

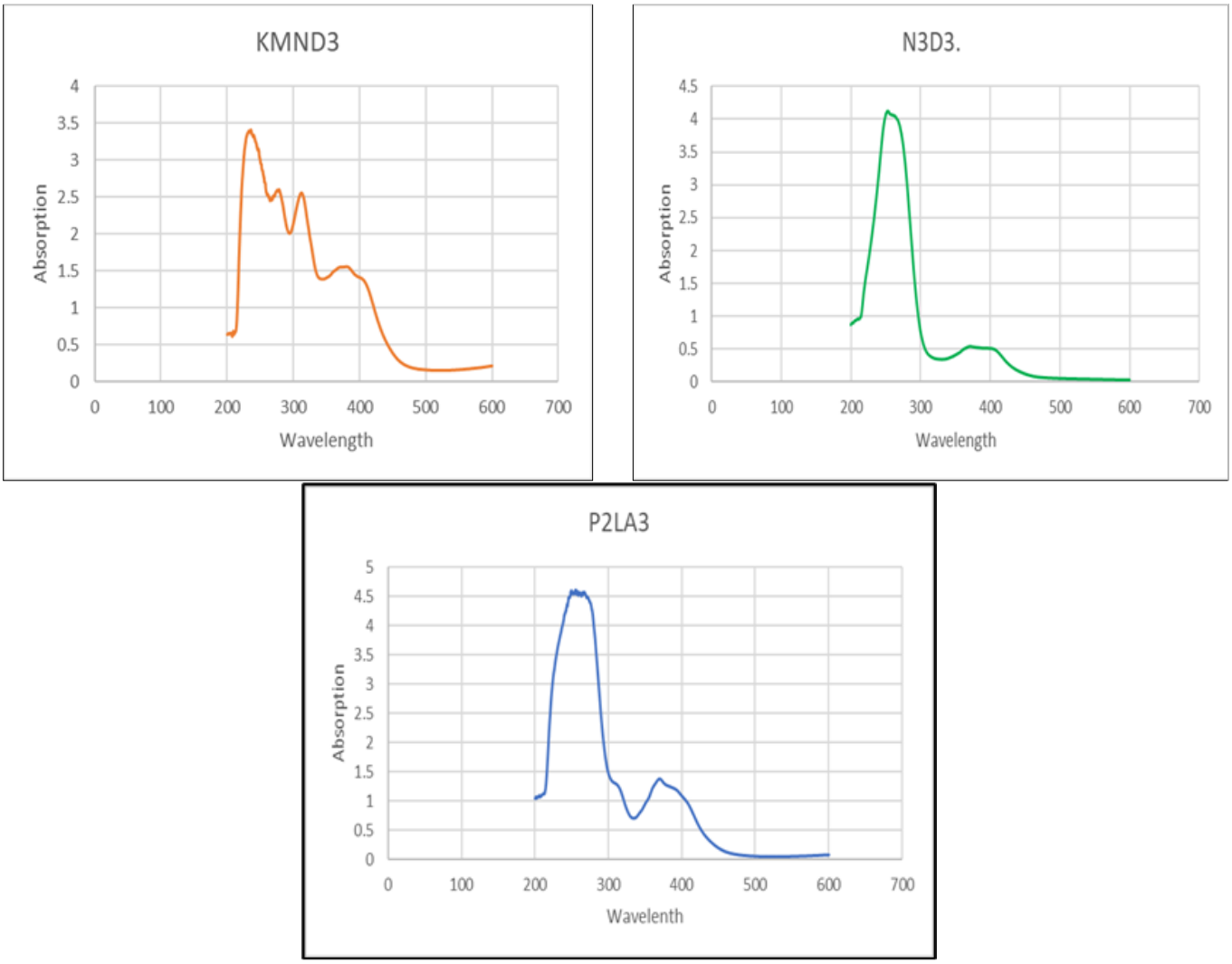

Fig. 2. UV-visible absorption spectrum for siderophore production. Peak at or near 404±10 shows siderophore production.

µM), followed by P2LA3 (54 µM) and N3D3 (27 µM), respectively. Compared to bacteria cultivated in Kings B Medium, those cultured in minimal medium succinate (MMS) produced more siderophores (25). A typical succinate medium achieved a high production of siderophores (17). *P. fluorescens* produced siderophores using the same medium (27). The carbon source used for growth plays a crucial role in pyoverdin synthesis also, succinate is a chromogenic substrate that increases siderophore production. The iron-deficient condition of the succinate medium induces siderophore yield. A study on the impact of media preparation for siderophore production revealed that only Succinate medium formed a green colour and a change in pH, not nutritional broth (28).

In-vitro antogonism

KMND3 exhibited the highest level of antibiosis, while N3D3 showed the lowest. Growth of *A. flavus* is maximum reduced by KMND3 (Table 1). Clear inhibition zones are visible and three bacterial strains efficiently suppress the fungal growth in a dual culture experiment (Fig. 3). N3D3 inhibited R. *solani* more than KMND3 and P2LA3. P. *aphanidermatum* and F. *solani* could not grow due to interference from P. *aeruginosa* culture filtrate (29). So, seed treatments are essential to boosting germination through disease reduction.

This pathogen can damage crop stands in chilly, moist soils, attack seedlings, induce damping off and even affect the crop later in the growth season. *T. asperellum* isolates revealed that it could stop R. *solani* growth in potato-dextrose agar medium, with a range of inhibition percentages varying from 59.57 % to 75.25 % (30). The mycelium of *F. oxysporum* and *R. solani* was lysed by *P. aeruginosa* strain PS-H-110 (31). Consider rephrasing as "For KMND3, fungal inhibition varied

Table 1. Statistical analysis for antibiosis by *P. aeruginosa* strains

Bacteria	Fungus				F-value (p-value)
	Fusarium oxYsporum	*Aspegillus flavus*	*Rhizactonia solani*	*Fusarium moniliforme*	
KMND3	18.64^{q}±5.794	26.21^{r}±2.47	15.82^{p}±6.59	18.61^{q}±5.93	53.87 (<.01)
N3D3	15.21^{p}±6.763	19.45^{q}±3.80	16.14^{p}±7.28	18.45^{q}±5.19	8.87 (<.01)
P2LA3	15.94^{p}±6.931	22.26^{r}±3.08	15.96^{p}±7.61	19.04^{q}±6.08	19.09 (<.01)

P,q and r are homogeneous groups by DMRT (p-value<.01).

Fig. 3. A dual culture experiment for an antibiosis study showed the successful growth inhibition of phytopathogenic fungi and their control plates.

significantly among species at the 1 % level of significance (F=53.87, p-value < 0.01). The highest inhibition is in *Aspergillus flavus,* whereas the lowest is in *R.solani*. The bacteria also reduced *F. moniliforme* and *F. oxysporum* in the dual culture. A biocontrol experiment with *P. aeruginosa* and F. *oxysporium* (32). Isolate BA5 showed the most potent inhibitory effect against Fusarium, with a mycelial reduction in the growth of 58.33 %.

The pathogen was effectively opposed by the isolate BA5, which also produced a variety of antagonistic chemicals, such as siderophores and volatile organic compounds. According to studies, *P.aeruginosa* strain HB9 is the strongest against *F. oxysporium* activity (33). The antifungal substance 2,4-diacetyl phloroglucinol (DAPG), produced by P. *fluorescence*, inhibited the growth of *f. moniliforme* mycelial cells (34). Among the strains of *Pseudomonas flourescence* Bandi 6 (29 mm) and Bandi 11 (28 mm) displayed the largest zone of inhibition against *Fusarium* sp. and Strain BG6 demonstrated superior activity against *Aspergillus* species (35).

In bacteria P2LA3, there is a significant difference in inhibition among fungi at a 1 % level of significance (F=19.09, p-value<.01). The highest inhibition in *Aspergillus flavu*s is lowest in *Fusarium oxysporum.* In bacteria N3D3, There is a significant difference in inhibition among fungi at a 1 % level of significance (F=8.87, p-value<.01). The highest inhibition is in *Aspergillus flavus,* lowest in *Fusarium oxysporum*. It is evident that the three bacterial strains efficiently reduced all four fungal pathogens. *Fusarium oxysporum* significantly reduced yield. In Kerala, prevalent fungi linked to sesamum seeds were *Aspergillus flavus*, A. *niger* and M*ucor haemali* (7). So, the three P.aeuginosa strains can be used as biocontrol agents in sesamum.

P. aerugenosa used as a bio-control agent in field experiments is scarce (36). Due to the generation of siderophores, *P. aeruginosa* strains KMND3, P2LA3 and N3D3 have demonstrated vigorous antifungal activity in the growth inhibition test. Purified siderophores and Pseudomonas cultures exhibit intense antifungal action against plant-destructive fungi, including *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Fusarium oxysporum* and *Sclerotium rolfsi*i, according to in vitro studies (37). Plant rhizosphere soil -derived P. *aeruginosa* strains, such as *P. aeruginosa* 7NSK2 from barley rhizosphere and P. *aeruginosa* PNA1 from chickpea rhizosphere, have been employed as efficient biocontrol agents in recent years (38,39). *A. flavus* and *F. oxysporum, F. moniliforme and R. solani* were significantly suppressed by the inhibitory volatile component generated by the isolate. Volatile chemicals have also been documented to play a part in other plant interactions. Laboratory studies have shown that biocontrol is feasible; nevertheless, the complicated environment around us must be considered when interpreting the possible consequences of hostile relationships.

Effect of different growth factors on siderophore production

The statistical analysis of the concentration of siderophores produced by KMND3 in the influence of different growth parameters and the mean difference is significant at the 0.5 level (Fig. 4). KMND3 displayed a diverse reaction to the presence of various sugars and starch supported increased growth and siderophore production to 71.82 µM (Fig. 4A). Fructose as a carbon source also supported siderophore increase by 44.53 µM. 18.85 µM yield obtained with sucrose. 0.5M sucrose increased siderophore production, while >5 µM of iron did not induce production in *Streptomyces fulvissimus* (40). The highest siderophore production was observed at about 31.59 % when the sucrose concentration was 15 g/L added (41).

In the treatment with Heavy metals (Fig. 4B), siderophore production quenched, only $CdCl_2$ showed a 10.22 µM siderophore production among heavy metals and $HgCl_2$ completely suppressed siderophore synthesis (6 µM), $MnCl_2$ inclusion in the medium resulted in 8.93 µM siderophore. Media treated with Pb^{2+}, Mn^{2+} and Mg^{2+} demonstrated culture growth and siderophore synthesis (42). Strong bioinoculants are thought to be rhizobacteria that stimulate plant growth and may flourish in the presence of various heavy metals (43). It has also been discovered that siderophores form complexes with iron and heavy metals such as nickel, cadmium, lead, arsenic (III, V), strontium, zinc, copper, magnesium, cobalt and aluminium (44). ZGKD3 strain exposed to several metals, including Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Mn^{2+} decreased production of siderophore and pyoverdine in the order: Zn^{2+}>Cd^{2+}>Mn^{2+}>Pb^{2+}>Ni^{2+}> Zn^{2+}>Cu^{2+}andCd^{2+}>Pb^{2+}>Mn^{2+}>Zn^{2+}>Cu^{2+}, respectively (45). For extracting and recovering Cd, Cu and Pb from contaminated water, *P. aeruginosa* PU21 biomass seems to be a useful bio-adsorbent (46). According to the findings, KMND3 can thrive in soil contaminated with Cadmium, Manganese and mercuric chloride, lowering their concentration and toxicity. This may make plants more resilient to cadmium toxicity and may be able to increase the effectiveness of phytoremediation in soil contaminated with Cd and Mn. The amount of siderophores reduced when the concentration of $FeCl_3$ increased in the medium (Fig. 4C). So, in the presence of 25 µM Fe+, irons gained an optimum level for siderophore production of about 122.90 µM. When *P. aeruginosa* KMND3 was exposed to 100 µM Fe in the medium, siderophore production was inhibited. The ideal range of ferric oxide concentration for bacterial growth is between 10^{-6} and 10^{-7} (47).

Iron concentration is essential for siderophore synthesis in situations where iron is scarce. According to statistical analysis, siderophore synthesis and iron content have a positive connection. Correlation between iron concentration and siderophore production in KMND3 (r^2= 0.70) r^2 is the coefficient of determination. Increased iron concentration negatively influences siderophore production in KMND3 (Fig. 5). Higher siderophore production would be induced by a lower iron concentration in the medium for the cell to bind to Fe^{3+} ions and make them available (48). In our study also, when the iron level goes above a certain point, however, siderophore production drops sharply. Due to the dominant role of an oxidative condition with an elevated pH in soil, Fe oxide formation is enabled, which reduces Fes' bio -availability. Iron reactivity and solubility are significantly affected by pH and nitrogen sources. Iron may oxidize

chemically spontaneously rather quickly in a neutral pH.

Of all the nitrogen sources investigated (Fig. 4D), yeast extract produced siderophore rates of 99.63 µM: Peptone (15.12 µM) and potassium (9.53µM) induced siderophore production. Nitrogen sources tested in an experiment conducted by (42), the optimum siderophore yields 104.8 µM were obtained in yeast extract. The optimum pH level for siderophore production was in the neutral pH of 61.23 µM (Fig. 4E). In low pH 5, siderophore production reduced to 19.62 µM and a high pH of 9 also reduced production. Additionally, another study reports that pH 7.0 (93.13 % SU) and 9.0 (90.47 % SU) produced the most siderophores (49). At a lower pH range (3.0-5.0), the *P. fluorescent* strain identified from the chickpea rhizosphere generated siderophores, with peaks at 385 nm, while at pH 7.0, the prominent peak was located at 410 nm (50). The pyoverdin spectra showed single peaks at higher pH values (pH 7). However, investigators stated that normal pyoverdin displayed multiple peaks at low pH values (51). According to reports, the absorption maxima of pyoverdin shifts to the lower ultraviolet region as the pH decreases. At pH 7, *P. asplenii* has an absorption maximum of approximately 407 nm, which shifts to 406 nm at pH 4.0 and 405 nm at pH 3.5 (52).In the current study, ideal conditions for growth for siderophore formation in *P. aeruginosa* ON329825 were a pH of 7, a Fe concentration of 25µM, starch as a carbon source and yeast as a nitrogen source.

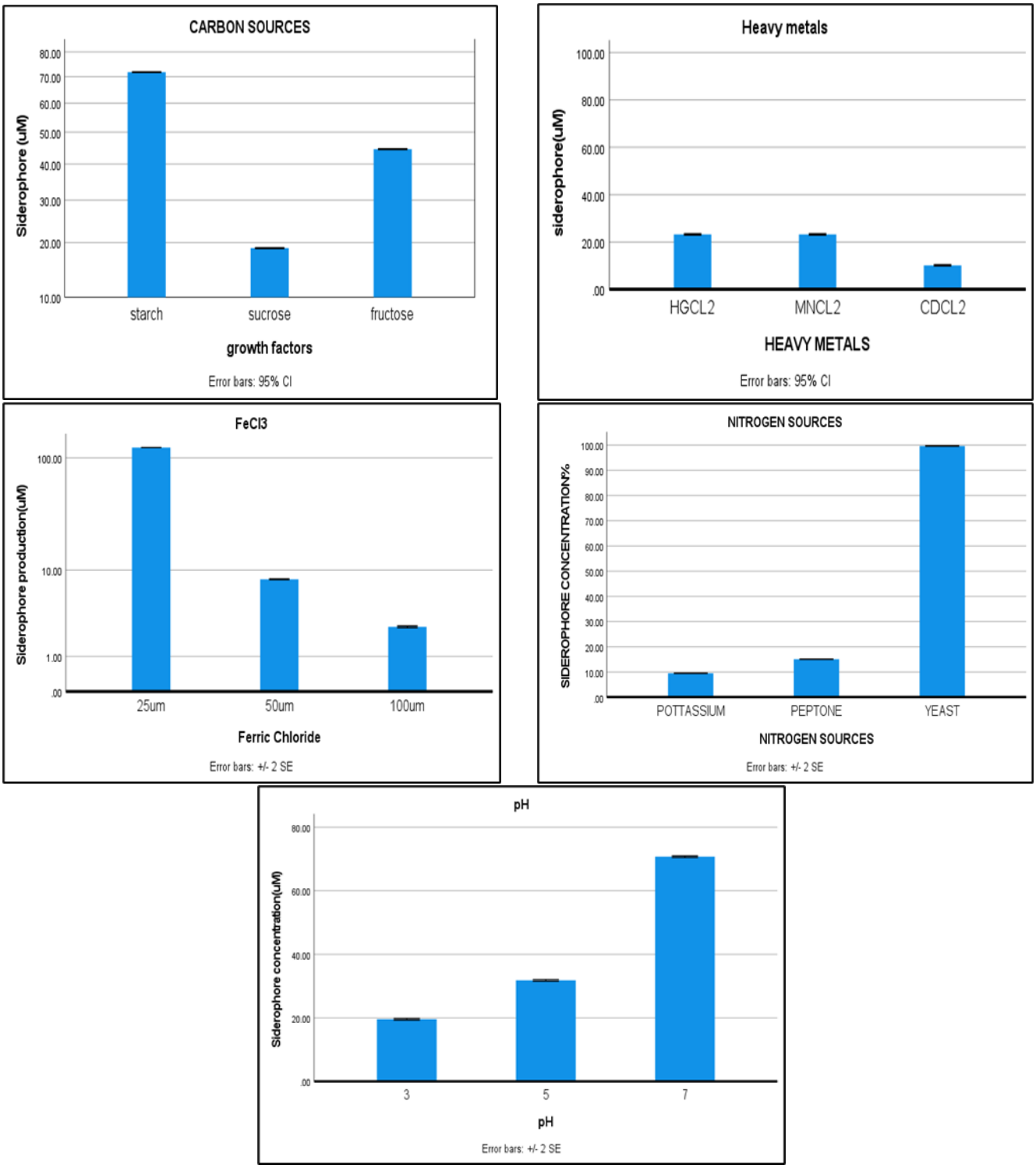

Fig. 4. Effect of growth factors on siderophore production: (A) Carbon; (B) Heavy Metals (C) $FeCl_3$ (D) Nitrogen source (E) pH

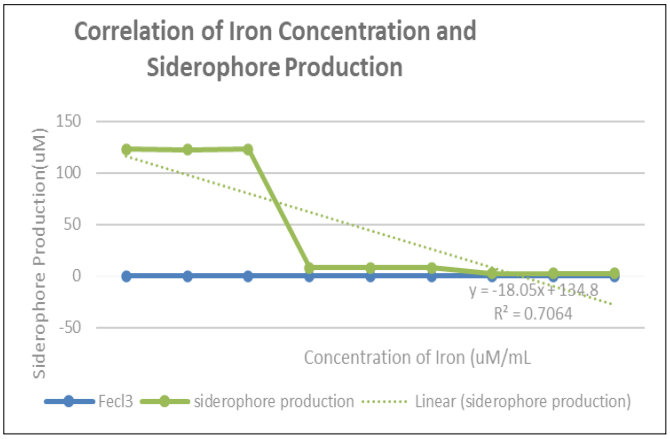

Fig. 5. Higher siderophore production in KMND3 induced by a lower concentration of iron in the medium.

Conclusion

Preliminary tests indicate that *P. aeruginosa* possesses PGPR features. This study identifies it as a potential siderophore-producing bacterium with significant biocontrol activity against phytopathogenic fungi. *P. aeruginosa* demonstrated multiple traits supporting plant growth and development, directly and indirectly contributing to plant health. An experiment was conducted to optimize six factors for improved siderophore production, including growth media, pH values, ferrous content, nitrogen and carbon sources and heavy metals' influence.

The most significant contributing elements to the enhanced synthesis of siderophores were yeast, starch, neutral pH and low iron content. For *P. aeruginosa* to produce siderophores, the optimal conditions for the increased absorption peak at pH 7, Fe concentration of 25 µM, presence of yeast and starch. Also, *P. aeruginosa* KMND3 (ON329825) exhibited a broad spectrum of hostile activities against *Aspergillus flavus, Fusarium moniliforme, Fusarium oxysporum and Rhizoctonia solani.* The three bacteria isolated from Onattukara, namely KMND3, N3D3 and P2LA3, showed significant siderophore production and antibiosis against the four major fungal pathogens already reported in sesame.

To ensure the success of Plant Growth-Promoting Rhizobacteria (PGPR), it is essential to understand their growth-promoting activities and their ability to counteract phytopathogens. Utilizing antagonistic bacteria for biological control is a safe and environmentally friendly approach. As far as we know, this is the first report documenting bacteria that produce siderophores capable of effectively suppressing the growth of significant phytopathogens in Onattukara Sesamum fields. *Pseudomonas aeruginosa* presents a practical, long-term solution for reducing economic losses caused by fungal diseases in soybeans. We are encouraged to include various plant varieties in our upcoming studies to explore the role of this PGPR concerning crop research. Therefore, understanding both the growth-promoting activities and antagonistic potential is key to developing an effective PGPR. The findings from this study are relevant to sustainable farming practices and have demonstrated statistical significance.

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Authors' contributions

KTP did experimental work and research article writing. CD developed an idea, proofread it and provided advice and guidance throughout the work.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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