SMTCT
Society of Land Measurements and Cadastre from Transylvania

Qessaoui R *et al.* (2024)
Notulae Scientia Biologicae
Volume 16, Issue 1, Article number 11555
DOI:10.15835/nsb16111555
Research Article

NSB
Notulae Scientia Biologicae

Pseudomonas siderophores: production, spectrophotometry detection and *Botrytis* suppression

Redouan QESSAOUI[1]*, Mohamed ELAALAOUI[2], Salahddine CHAFIKI[1], Mohamed ALOUANI[3], Naima CHABBI[1], Naima AIT ABD[1], Abdelghani TAHIRI[1], Rachid BOUHARROUD[1]

[1]*Regional Center of Agricultural Research of Agadir, National Institute of Agricultural Research (INRA), Avenue Ennasr, B.P. 415 Rabat Principale, Rabat 10090, Morocco; redouan.qessaoui@inra.ma (*corresponding author); salahchafiki0@gmail.com; chabbinaima71@gmail.com; naima.aitaabd@inra.ma; abdelghani.tahiri@inra.ma; rachid.bouharroud@inra.ma*
[2]*Regional Center of Agricultural Research of Settat, National Institute of Agricultural Research (INRA), Avenue Ennasr, B.P. 415 Rabat Principale, Rabat 10090, Morocco; mohamed.elaalaoui@inra.ma*
[3]*Ibn Zohr University, Faculty of Sciences, Laboratory of Biotechnologies and Valorization of Natural Resources, Agadir, Morocco; m.alouani@uiz.ac.ma*

Abstract

Siderophores are iron-chelating agents produced by almost all microorganisms in response to iron deficiency. Due to the requirement of iron for cell growth and metabolism, siderophore-mediated acquisition of iron plays a central role in determining the ability of different microorganisms to colonize plant roots and contributes to microbial interactions in the plant rhizosphere. In this study, five new *Pseudomonas* (Q14B, Q13B, Q7B, Q6B, Q1B), isolated from the rhizosphere of tomato in Morocco, were examined for siderophores production capacity. The results show that all five isolates produced siderophores on both solid and liquid mediums. In liquid medium, the highest level of production is obtained by Q13B (53.8%). Concerning siderophores' chemical types, the five strains of *Pseudomonas* produce two types of siderophores hydroxamate and catecholate. It was shown by the peaks of absorbance in the wavelength 495 and between 420-450 nm for catecholate and hydroxamate-type siderophores respectively. The results showed that the production of siderophores is progressively inhibited with increasing concentrations of iron in the medium. The maximum production was obtained with a concentration of 0.5 µM, while the lowest was recorded at 10.0 µM of iron. The results of this study showed that the five *Pseudomonas* isolates producing siderophores could be potential biological control agents.

Keywords: catecholate; hydroxamate; *Pseudomonas*; siderophores

Introduction

Iron (Fe) is an essential nutrient for all organisms (Miethke and Marahiel, 2007). As a transition element, iron can adopt either of two ionic forms, reduced ferrous (Fe^{2+}) or oxidized ferric (Fe^{3+}). Also, it is the

Received: 20 Apr 2023. Received in revised form: 10 Jan 2024. Accepted: 12 Mar 2024. Published online: 22 Mar 2024.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

major redox mediator in biology because of its capacity to exchange electrons (Haas *et al*., 2008). In addition, this nutrient is mainly involved in the growth of almost all living microorganisms, acting as a catalyst in oxygen metabolism, enzymatic processes, electron transfer, and DNA and RNA synthesis (Aguado-Santacruz *et al.* 2012). Furthermore, iron deficiency in crops results in iron chlorosis, making them micronutrient deficient and hence sensitive to microbial infections (Sayyed *et al*., 2013). Under Fe-limiting conditions, many microorganisms and plants produce ferric iron chelators, which solubilize and transport Fe (III) into the cell, making it assimilable by plants in Fe^{2+} form (Ongena *et al*., 2002). These secondary metabolites are called siderophores. They are divided into three key families depending on the characteristic functional group, i.e., hydroxamates, catecholate/phenolates, and carboxylates (Schwyn and Neilands, 1987; Sandy and Butler, 2009; Sayyed *et al*., 2019). The formation of Fe (III) siderophore complexes is affected by pH because of the competition for the free siderophore ligands between free protons and Fe (Albrecht-Gary and Crumbliss, 1998). Siderophores produced by PGPR play an important role in plant growth promotion and prevent plant pathogens from iron nutrition (Kloepper *et al*., 1980; Shaikh and Sayyed, 2015; Saha *et al*., 2015; Sayyed *et al*., 2019). Many studies have reported that the *Pseudomonas* genus was known for its ability to synthesize siderophores and to reduce the incidence of plant diseases inhibiting the growth of many plant pathogens (Qessaoui *et al*., 2021; Ghssein and Ezzeddine, 2022; Saranraj *et al*., 2023). This inhibition capacity can be explained by several mechanisms including the production of a wide range of secondary metabolites that have inhibitory effects against plant pathogens (Qessaoui *et al*., 2021, 2022). The objective of this study is to evaluate the effect of the five Siderophore Producing *Pseudomonas* strains, against *Botrytis cinerea.*

Materials and Methods

Bacterial isolates

The five *Pseudomonas* strains (Q14B, Q13B, Q7B, Q6B, Q1B) were isolated from the rhizosphere of tomato at the experimental farm of the National Institute for Agricultural Research in Belfaa-Souss-Massa region (30°02′42.2"N 9°33′13.4"W) and were previously characterized based on a partial rpoD gene sequence using the primers PsrpoD FNP1 and PsrpoDnprpcr1 (Qessaoui *et al*., 2019).

Siderophores qualitative production

Siderophore production by the different isolates was qualitatively tested using Chrome Azurol sulfonate medium (CAS-medium) described by Schwyn and Neilands (1987). Each fluorescent *Pseudomonas* isolate was streaked on the surface of CAS agar medium and incubated at 28 °C for 3 days. After the incubation, siderophore production was confirmed by a yellow-orange halo around the colonies. The assay was carried out in triplicates.

Siderophores quantitative production

Quantitative analysis of siderophore production was done on King B liquid medium inoculated with 100 µL *Pseudomonas* isolate culture (10^8cfu/ml) and incubated at 28 °C for 72 hours. Cultures were centrifuged at 5000 rpm for 30 min, and 500 µL of the supernatant was mixed with 500 µL CAS solution. The color changed from blue to orange indicating siderophore production. After 20 min of incubation, optical density was measured by the Optizen 3220UV Double Beam UV-Vis spectrophotometer (Mecasys, Korea) at 630 nm. The percentage of siderophore was calculated in terms of % of siderophore units using the following formula:

$$\%\ Siderophores\ units = \frac{RA - SA}{RA} * 100 \quad (1)$$

RA means absorbance of reference (CAS reagent) and SA means absorbance of the sample (Pastor *et al*., 2014).

Chemical nature determination of the siderophores produced by the five strains

The supernatants obtained to quantify the siderophores were sterilized by filtration (0.22 µm) and subjected to chemical analysis to determine the nature of the siderophores produced. $FeCl_3$ test was used to determine Hydroxamates and catecholate (Neilands, 1981).

Catecholates

To determine catecholate Siderophore, 1 ml of $FeCl_3$ (2%) was added to 1 ml of filtrate. A maximum absorbance at 495 nm indicated the presence of catecholate-type ferri-siderophores (Neilands, 1981).

Hydroxamates

1 ml of 2% ferric chloride solution was added to 1 ml of culture filtrate. The formation of red or purple color indicated the presence of siderophores. A maximum absorbance between 420 and 450 nm of ferrated siderophores indicated its hydroxamate nature (Neilands, 1981). The peak was noted on a UV–visible spectrophotometer.

Effect of iron concentrations on the production of siderophores

To determine the effect of the iron concentration on the biosynthesis of siderophores in the five *Pseudomonas,* six iron concentrations ($FeCl_3$. $6H_2O$) were tested (0.5; 1; 1.5; 2.5; 5, and 10 µM). The five *Pseudomonas* strains were cultured in King B liquid medium, flooded with different concentrations of iron with three repetitions for each concentration. After incubation at 28 ± 2 °C for 72 h at 120 rpm, the percentage of siderophores is calculated using the formula described by Pal and Gokarn (2010). To remove traces of iron, all the glassware used was cleaned with 6 M HCl and washed with double-distilled water.

Antifungal activity of the five Pseudomonas strains against Botrytis cinerea

In vitro evaluation of five *Pseudomonas* strains was conducted using a dual culture technique on PDA (Kaur *et al*., 2013). A heavy inoculum of the individual isolate was applied as a band of 1.5 cm length equidistantly on three opposite edges of the agar medium in the Petri plate using an inoculation loop. A mycelial disc of 5 mm diameter from 7-day-old culture of *B. Cinerea* was placed at the center of the Petri plate. Three replications were maintained for an isolate. Plates containing the pathogen alone served as control. The inoculated plates were incubated at 25 °C for five days. After the incubation period, the mycelial growth of *B. Cinerea* was recorded, and the mycelial growth inhibition percentage (MGIP) was calculated using the following formula:

$$\% \text{ MGIP} = \frac{\text{r1} - \text{r2}}{\text{r1}} * 100 \qquad (2)$$

r1 is the radial growth of the fungus in the control and r2 is the radial growth of the fungus in the treated plates (Chaurasia *et al*., 2005; Hummadi *et al*., 2022).

Statistical analysis

Data analysis was subjected to ANOVA using Statistica software ver.6. Data for Siderophore production are presented as means ± standard deviation. Any difference mentioned is significant at $p < 0.01$ using the Newman–Keuls test

Results

Siderophores production

The five *Pseudomonas* strains were tested for siderophore production capacity. All these isolates produced siderophores in both solid and liquid media. This production is shown by the presence of yellow-orange halo around the colony (Figure 1) in solid medium with a diameter ranging from 4 to 6 mm. The color change between yellow and orange indicated the production of different types of siderophores.

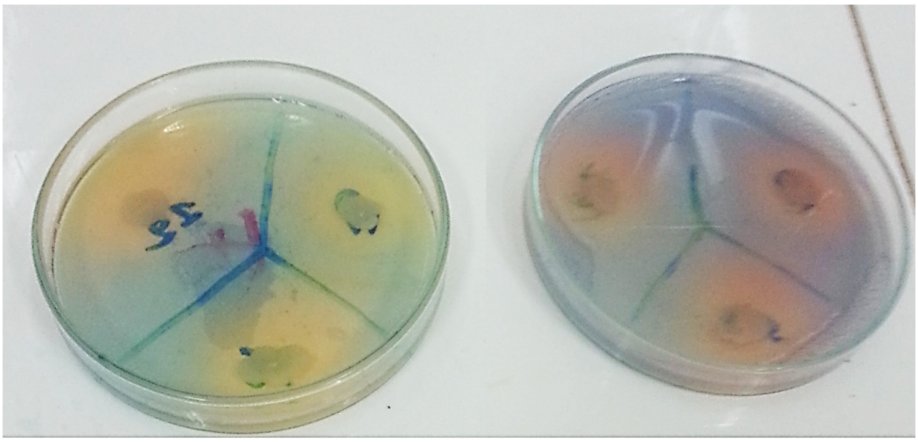

Figure 1. The appearance of orange color and zone formation indicating siderophore production in CAS agar plate assay

In liquid medium, the percentage of siderophore production varied according to the strain of *Pseudomonas* tested. The highest level of production is noted for Q13B (53.8%) (Figure 2). However, Q1B showed the lowest one (17.5%).

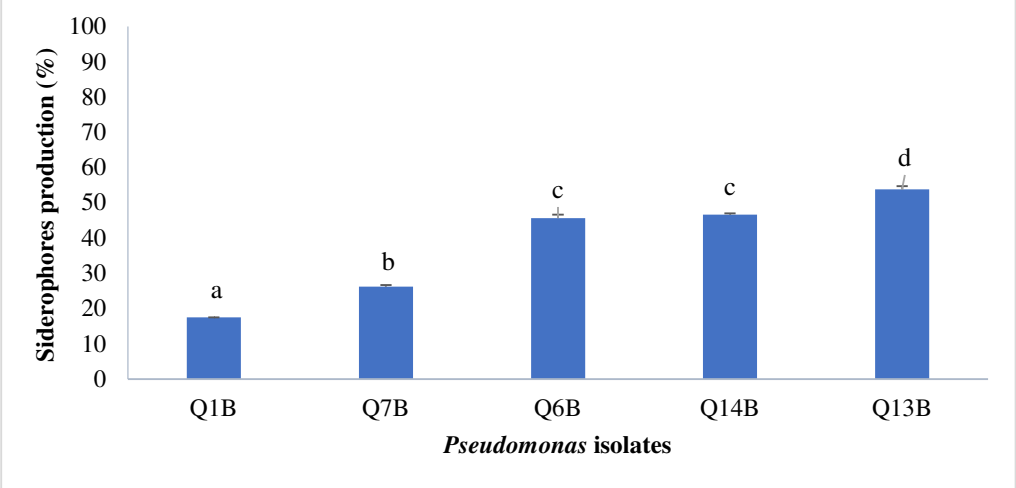

Figure 2. Siderophores production by five *Pseudomonas* in liquid medium

Chemical nature determination of the siderophores produced by the five strains

The five strains of *Pseudomonas* produce two types of siderophores; hydroxamates, and catecholates. This production was shown by the peaks of absorbance in the wavelength (495 nm) for the catecholate-type

siderophore (Figure 3). A maximum absorbance between 420-450 nm indicated the presence of hydroxamate-type siderophores (Figure 4).

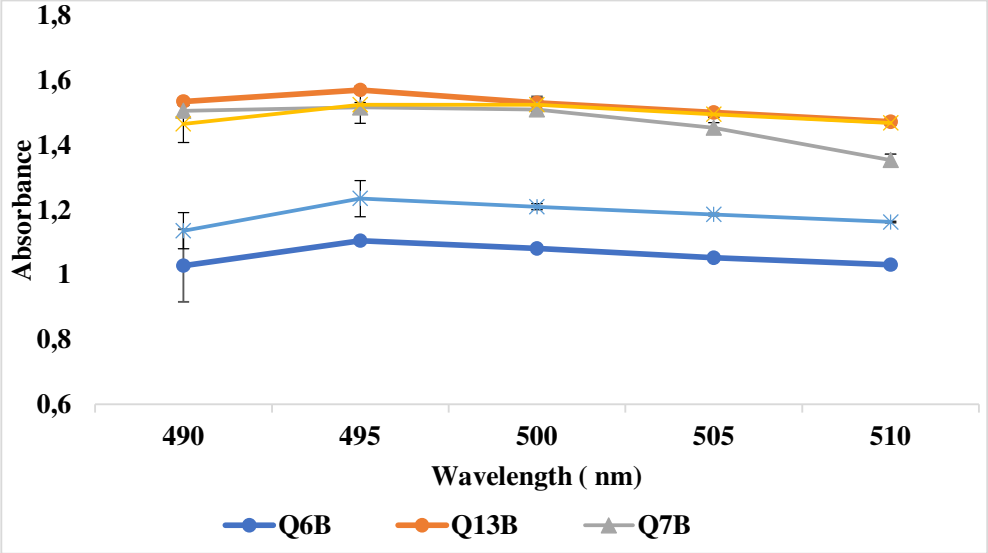

Figure 3. Detection of the chemical nature of siderophores produced by *Pseudomonas* strains (case of catecholates)

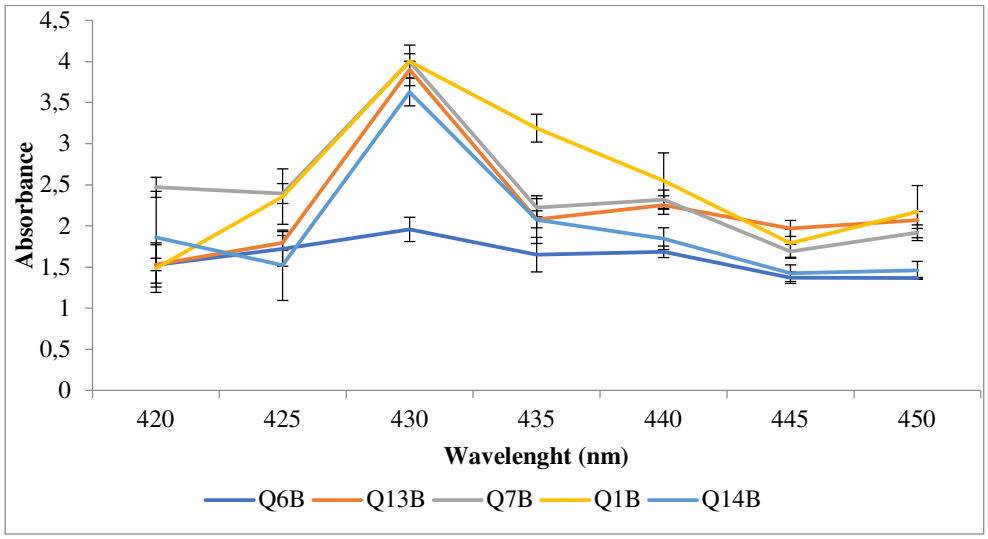

Figure 4. Detection of the chemical nature of siderophores produced by *Pseudomonas* strains (case of hydroxamates)

Effect of iron concentrations on the production of siderophores

The results obtained showed that the production of siderophores is progressively inhibited with increasing concentrations of iron (0.5, 1, 1.5, 2.5, 5, 10 µM $FeCl_3$) (Table 1). The maximum productions of siderophores for all isolates studied were obtained with a concentration of 0.5 µM of iron, while the lowest was recorded at 10.0 µM of $FeCl_3$ (Table 1). The difference in siderophore production is significant between the five *Pseudomonas* strains at $FeCl_3$ concentrations greater than or equal to 1.5 µM.

Table 1. Effect of iron concentration on the production of siderophores by the five *Pseudomonas* strains

	Iron concentrations					
	0.5 µM	**1 Mm**	**1.5 Mm**	**2.5 Mm**	**5 Mm**	**10 Mm**
Q14B	84.03±2.71^{a}	77.39±0.09^{a}	55.18±0.06^{c}	53.91±0.07^{b}	50.88±0.03^{a}	28.09±0.06^{c}
Q13B	76.81±0.07^{a}	73.89±0.06^{a}	71.09±0.03^{c}	66.21±0.03^{d}	51.65±0.03^{a}	49.79±0.03^{a}
Q7B	79.22±4.10^{a}	77.37±4.2^{a}	66.05±2.48^{a}	65.10±0.66^{c}	59.18±4.7^{b}	49.38±0.96^{a}
Q6B	83.79±0.03^{a}	79.96±0.03^{a}	73.85±0.03^{d}	73.29±0.03^{c}	71.52±0.14^{c}	66.69±0.41^{c}
Q1B	80.84±3.49^{a}	79.07±3.53^{a}	53.04±0.68^{b}	52.12±1.00^{a}	51.11±0.00^{a}	50.88±0.03^{d}

*By rows, values with the same letters are not significantly different according to the Newman & Keuls test at 1%. Values are the average of three replications; values after ± represent the standard deviation

Antifungal activity by the five Pseudomonas strains against Botrytis cinerea

The result obtained for the antifungal activity of *Pseudomonas* strains tested showed that all five strains (Q14B, Q13B, Q7B, Q6B, Q1B) inhibited mycelial growth of *B. cinerea* (Figure 5 and 6). Mycelial growth inhibited an average of 40.42% to 63.26% for Q6B and Q1B respectively compared to the control. The two Q7B and Q1B showed potent inhibition of *B. cinerea*, above 60% (Figure 6).

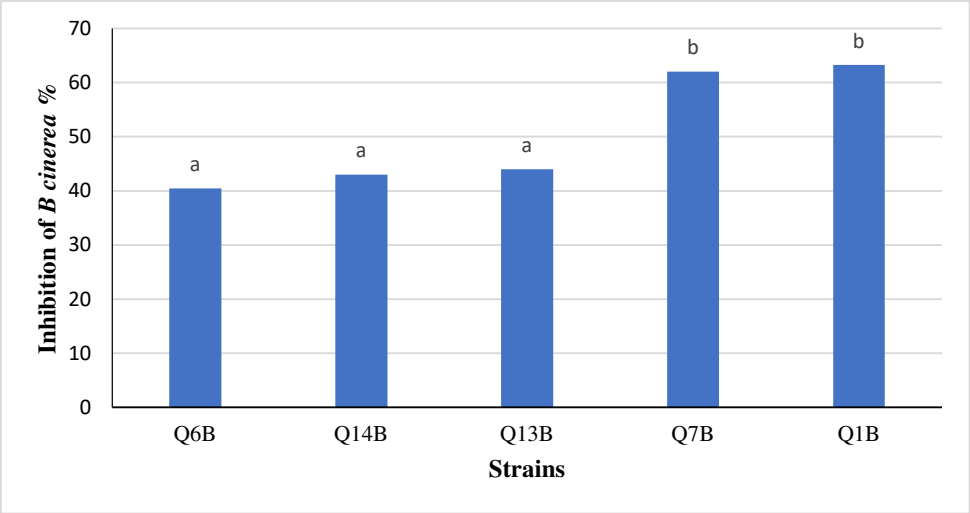

Figure 5. Effect of five *Pseudomonas* stains against *B. cinerea*
Bars with the same letters are not significantly different at P<0. 01 using the Newman-keuls test

Figure 6. Inhibition of mycelium growth of *B. cinerea* by *Pseudomonas* strains in PDA medium

Discussion

In the present study, the tests regarding the production of siderophores are carried out on King B medium which is known by its appropriate iron composition allowing the release of siderophores into the medium by bacteria. The quantitative estimation of siderophore production on CAS liquid medium is determined after 5 days of incubation at 30 °C. Analysis of variance reveals a difference in efficacy ($p < 0.01$) among isolates in terms of their ability to produce siderophores. The production percentages range from 17.47 to 58.88%, with Q6B, Q14B, and Q13B being the most efficient strains. The lowest percentage has been observed for Q1B. For qualitative estimation, the appearance of an orange halo formed around the colony on a solid CAS medium indicated the production of siderophores by the bacteria. It should be noted that the liquid medium is more suitable for siderophore production by the bacteria. All the strains tested in the current study produced two kinds of siderophores: hydroxamates and catecholates. Many studies showed that Hydroxamates are produced by both bacteria and fungi, while catecholates are produced exclusively by bacteria, and include two groups, catechol, and hydroxyl with a ligand function (Baakza *et al*., 2004). Another kind of siderophore is carboxylates, these siderophores are produced by a fungal (mucosal) group, and very few bacteria, such as *Rhizobium meliloti* and *Staphylococcus hyicus*, and they are generally bound to iron by two groups, one hydroxyl and the other carboxyl (Drechsel *et al*., 1995; Baakza *et al*., 2004). By to detect this type of compound, several techniques based on chemical properties have been used including CAS medium (Schwyn and Neilands, 1987). All the isolates tested in this study produced hydroxamate and catecholate siderophores in the CAS medium. Many studies reported that siderophores are produced by both bacteria and fungi as well as some monocotyledons species only when the medium is deficient in iron (Crowley *et al*., 1991; Ratledge and Dover, 2003; Lemare *et al*., 2022; Meyer and Hornsperger, 1978). In this same context, Meyer and Abdallah (1978) reported that this product has also been synthesized by many strains of fluorescent *Pseudomonas* such as *P. aeruginosa*, *P. putida*, *P. chlororaphis*, *and P. aureofaciens*. To evaluate the influence of iron concentration on siderophore production, the highest siderophore production for all the strains studied was obtained with 0.5 μM $FeCl_3$ concentration, and the lowest was recorded at 10.0 μM $FeCl_3$. For all isolates tested, siderophore production is progressively inhibited with increasing iron concentrations. Similar results were obtained by Budzikiewicz (1993); Rachid *et al*. (2005) and Sayyed *et al*. (2005). Regarding the effect of incubation time on siderophore production, Sayyed *et al*. (2005) showed that the siderophores production started after 12 h of incubation. Siderophore maximum production was observed at the end of the logarithmic growth phase (Sharma and Johri, 2003).

This work highlights the importance of rhizospheric *Pseudomonas* in siderophores production. Our study further confirms the potential of *Pseudomonas* in *Botrytis* control. The use and application of these soil microorganisms in biological control can be an effective alternative to current practices and can reduce the use of harmful agrochemicals. Our research further indicates that microbial soil biodiversity can play an important role in disease plant protection and be utilized to reduce pesticide use. Future field studies should focus on field trials where these isolates can be used as a product to control *Botrytis cinerea* and stimulate plant growth.

Conclusions

This study investigates the siderophore production capacity of five new *Pseudomonas* strains isolated from the rhizosphere of tomato plants in Morocco. All five isolates demonstrated the ability to produce siderophores on both solid and liquid media. Strain Q13B exhibited the highest production level. Results revealed that these strains produced two types of siderophores: hydroxamates and catecholates. Furthermore, the production of siderophores was found to be inhibited by increasing the concentration of iron in the medium. These results indicate that the identified *Pseudomonas* isolates have the potential to act as biological

control agents against plant pathogens, including *Botrytis cinerea*. This study highlights the importance of rhizospheric Pseudomonas in siderophore production and their potential contribution to plant disease management, reducing the dependence on harmful agrochemicals.

Authors' Contributions

RQ, ME, SC and RB: Conceptualization, Preparation, Validation, Methodology Supervision. RQ, MA, NC, NAA, AT, and RB: Data analysis, Results validation, Writing-Reviewing and Editing.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aguado-Santacruz GA, Moreno-Gómez B, Jiménez-Francisco B, García-Moya E, Preciado-Ortiz RE (2012). Impact of the microbial siderophores and phytosiderophores on the iron assimilation by plants: a synthesis. Revista Fitotecnia Mexicana 35:9-21.

Albrecht-Gary AM, Crumbliss AL (1998). Coordination chemistry of siderophores: thermodynamics and kinetics of iron chelation and release. Metal Ions in Biological Systems 35:239-328.

Baakza A, Vala AK, Dave BP, Dube HC (2004). A comparative study of siderophore production by fungi from marine and terrestrial habitats. Journal of Experimental Marine Biology and Ecology 311:1-9. *https://doi.org/10.1016/J.JEMBE.2003.12.028*

Budzikiewicz H (1993). Secondary metabolites from fluorescent pseudomonads. FEMS Microbiology Letters 104:209-228. *https://doi.org/10.1016/0378-1097(93)90597-U*

Chaurasia B, Pandey A, Palni LMS, Trivedi P, Kumar B, Colvin N (2005). Diffusible and volatile compounds produced by an antagonistic Bacillus subtilis strain cause structural deformations in pathogenic fungi *in vitro*. Microbiological Research 160:75-81. *https://doi.org/10.1016/J.MICRES.2004.09.013*

Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ (1991). Mechanisms of iron acquisition from siderophores by microorganisms and plants. Iron Nutrition and Interactions in Plants 213-232. *https://doi.org/10.1007/978-94-011-3294-7_27*

Drechsel H, Tschierske M, Thieken A, Jung G, Zähner H, Winkelmann G (1995). The carboxylate type siderophore rhizoferrin and its analogs produced by directed fermentation. Journal of Industrial Microbiology 14:105-112. *https://doi.org/10.1007/BF01569891*

Ghssein G, Ezzeddine Z (2022). A review of *Pseudomonas aeruginosa* metallophores: Pyoverdine, pyochelin and pseudopaline. Biology (Basel) 11:1711. *https://doi.org/10.3390/biology11121711*

Haas H, Eisendle M, Turgeon BG (2008). Siderophores in fungal physiology and virulence. Annual Review of Phytopathology 46:149-187. *https://doi.org/10.1146/annurev.phyto.45.062806.094338*

Hummadi EH, Cetin Y, Demirbek M, Kardar NM, Khan S, Coates CJ, … Butt TM (2022). Antimicrobial volatiles of the insect pathogen *Metarhizium brunneum*. Journal of Fungi 8:326. *https://doi.org/10.3390/JOF8040326/S1*

Kaur T, Sharma D, Kaur A, Manhas RK (2013). Antagonistic and plant growth promoting activities of endophytic and soil actinomycetes. Archives of Phytopathology and Plant Protection 46:1756-1768. *https://doi.org/10.1080/03235408.2013.777169*

Kloepper JW, Leong J, Teintze M, Schroth MN (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885-886. *https://doi.org/10.1038/286885a0*

Lemare M, Puja H, David SR, Mathieu S, Ihiawakrim D, Geoffroy VA, Rigouin C (2022). Engineering siderophore production in Pseudomonas to improve asbestos weathering. Microbial Biotechnology 15:2351-2363. *https://doi.org/10.1111/1751-7915.14099*

Meyer JM, Abdallah MA (1978). The fluorescent pigment of *Pseudomonas fluorescens*: Biosynthesis, purification and physicochemical properties. Journal of General Microbiology 107:319-328. *https://doi.org/10.1099/00221287-107-2-319/CITE/REFWORKS*

Meyer JM, Hornsperger JM (1978). Role of pyoverdine(pf), the iron-binding fluorescent pigment of *Pseudomonas fluorescens,* in iron transport. Journal of General Microbiology 107:329-331. *https://doi.org/10.1099/00221287-107-2-329*

Miethke M, Marahiel MA (2007). Siderophore-based iron acquisition and pathogen control. Microbiology and Molecular Biology Reviews 71:413-451. *https://doi.org/10.1128/MMBR.00012-07*

Neilands JB (1981). Iron absorption and transport in microorganisms. Annual Reviews in Nutrition 1:27-46. *https://doi.org/10.1146/annurev.nu.01.070181.000331*

Ongena M, Giger A, Jacques P, Dommes J, Thonart P (2002). Study of bacterial determinants involved in the induction of systemic resistance in bean by *Pseudomonas putida* BTP1. European Journal of Plant Pathology 108:187-196. *https://doi.org/10.1023/A:1015141503755/METRICS*

Pal RB, Gokarn K (2010). Siderophores and pathogenicity of microorganisms. Journal of Bioscience and Technology 1:127-134.

Pastor N, Rosas S, Luna V, Rovera M (2014). Inoculation with *Pseudomonas putida* PCI2, a phosphate solubilizing rhizobacterium, stimulates the growth of tomato plants. Symbiosis 62:157-167. *https://doi.org/10.1007/s13199-014-0281-3*

Qessaoui R, Bouharroud R, Furze JN, El Aalaoui M, Akroud H, Amarraque A, … Chebli B (2019). Applications of new rhizobacteria *Pseudomonas* isolates in agroecology via fundamental processes complementing plant growth. Scientific Reports 9. *https://doi.org/10.1038/s41598-019-49216-8*

Qessaoui R, Lahmyed H, Ajerrar A, Furze JN, Timothy P, Alouani M, … Bouharroud R (2021). Solutions rhizosphériques: Isolats de *Pseudomonas* contre *Botrytis cinerea* de la tomate. African and Mediterranean Agricultural Journal Al Awamia, pp 50-72.

Qessaoui R, Zanzan M, Ajerrar A, Lahmyed H, Boumair A, Tahzima R, … Bouharroud R (2022). *Pseudomonas* isolates as potential biofungicides of green mold (*Penicillium digitatum*) on orange fruit. International Journal of Fruit Science 22:142-150. *https://doi.org/10.1080/15538362.2021.2018671*

Rachid D, Ahmed B (2005). Effect of iron and growth inhibitors on siderophores production by *Pseudomonas fluorescens*. African Journal of Biotechnology 4(7):697-702. *https://doi.org/10.5897/AJB2005.000-3129*

Ratledge C, Dover LG (2000). Iron metabolism in pathogenic bacteria. Annual Reviews in Microbiology 54(1):881-941. *https://doi.org/10.1146/ANNUREV.MICRO.54.1.881*

Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2015). Microbial siderophores and their potential applications: a review. Environmental Science and Pollution Research 23:3984-3999. *https://doi.org/10.1007/S11356-015-4294-0*

Sandy M, Butler A (2009). Microbial iron acquisition: Marine and terrestrial siderophores. Chemical Reviews 109:4580-4595. *https://doi.org/10.1021/CR9002787/ASSET/CR9002787.FP.PNG_V03*

Saranraj P, Sayyed RZ, Kokila M, Salomi V, Sivasakthivelan P, Manigandan M, Mawar R (2023). Evolving concepts of biocontrol of phytopathogens by endophytic *Pseudomonas fluorescence*. Plant Growth Promoting Microorganisms of Arid Region 365-388. *https://doi.org/10.1007/978-981-19-4124-5_17*

Sayyed RZ, Badgujar MD, Sonawane HM, Mhaske MM, Chincholkar SB (2005). Production of microbial iron chelators (siderophores) by fluorescent *Pseudomonas*. IJBT 4(4):484-490.

Sayyed RZ, Chincholkar SB, Reddy MS, Gangurde NS, Patel PR (2013). Siderophore producing PGPR for crop nutrition and phytopathogen suppression. Bacteria in Agrobiology: Disease Management 449-471. *https://doi.org/10.1007/978-3-642-33639-3_17/COVER*

Sayyed RZ, Seifi S, Patel PR, Shaikh SS, Jadhav HP, Enshasy H El (2019). Siderophore production in groundnut rhizosphere isolate, *Achromobacter* sp. RZS2 influenced by physicochemical factors and metal ions. Environmental Sustainability 2:117-124. *https://doi.org/10.1007/S42398-019-00070-4*

Schwyn B, Neilands JB (1987). Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160:47-56. *https://doi.org/10.1016/0003-2697(87)90612-9*

Shaikh SS, Sayyed RZ (2015). Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. Plant Microbes Symbiosis: Applied Facets 337-351. *https://doi.org/10.1007/978-81-322-2068-8_18/COVER*

Sharma A, Johri BN (2003). Growth promoting influence of siderophore-producing Pseudomonas strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. Microbiological Research 158:243-248. *https://doi.org/10.1078/0944-5013-00197*