Advances in Bioresearch
Adv. Biores., Special Issue 1: 2023: 345-351
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.S1.345351

Advances in Bioresearch

Characterization of Siderophore Produced by Bacterial Endophytes Isolated from Aquatic Weed Plant

Shivangi H Zaveri[1*], Sumita Dasgupta[2], Piyush Desai[3]
[1] PhD research scholar, Department of Biotechnology, Bhagwan Mahavir Center for Advance Research, Bhagwan Mahavir University, Surat 395007, Gujarat, India
[2] Assistant professor, Department of Biotechnology, Bhagwan Mahavir College of Basic and Applied Sciences, Bhagwan Mahavir University, Surat 395007, Gujarat, India
[3] Dean, Science Faculty, Bhagwan Mahavir University, Surat, Gujarat, India.
[1*]Corresponding author Email ID: zaverishivangi@gmail.com

ABSTRACT
Siderophore are organic compound of low molecular weight (<10kDa), possessing high specific affinity to chelate iron. In the present study bacterial endophytic strains were isolated from aquatic weed plant. Total 10 isolates were obtained, 05 isolates were from root (designated as ECR1, ECR2, ECR3, ECR4, and ECR5) and 05 isolates were from leaf (designated as ECL1, ECL2, ECL3, ECL4, and ECL5).All isolates from root showed positive results for siderophore production. Qualitative and quantitative characterization of siderophore was conducted and tests were performed for determining the type of siderophore. As per qualitative results, it was found that ECR1, ECR2 and ECR4 were siderophore of catecholate type and ECR3 and ECR5 were of hydroxamate type. As per quantitative results, the percentage of siderophore production was in range of 34% to 56%. Further studies are necessary in order to determine the plant growth promoting role of siderophore produced by the isolated strains.
KEYWORDS: Siderophore, *Eichhornia crassipes*, CAS agar assay, Hydroxamate, Catecholate

Received 24.05.2023 Revised 01.07.2023 Accepted 23.08.2023

How to cite this article:
Nehal C, Richa S, Hiral G, Vidhi S, and Tarun Y Association between processing method and Glycemic Indices of South Indian food.. Adv. Biores., Special 1:2023: 345-351.

INTRODUCTION

Endophytes which can be fungi or bacteria live inside plant tissues without harming their host (1). They benefit from their host's protection and nutrition, which helps the host plant thrive. The coordinated activity of phytohormone production, biological nitrogen fixation, phosphate solubilization, modulation of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase expression, production of siderophores, and biocontrol activities is what causes endophytes to promote plant growth. Among these, the formation of siderophore by endophytes aids plants in the storage of iron and raises the concentration of bioavailable iron in inner tissues (2).

After oxygen, silicon, and aluminum, iron is the element with the fourth-highest abundance in the crust of the earth. It is essential for the growth of living things because it works with enzymes to carry out several metabolic activities (3). Despite its abundance, it is essentially inaccessible to microorganisms and plants. Even certain plants and soil microbes have developed methods to maintain iron availability in environments where iron "Fe3+" is only present at concentrations of 10–18 M and is not soluble at physiological pH. This tactic entails the production of siderophores, low molecular weight iron chelating substances with a wide range of structural variations that can be categorized according to their primary chelating groups (4). Microorganisms have developed specific mechanism for uptake of iron by releasing siderophores to scavenge iron (5). Siderophores are high-affinity ferric ion-specific chelators with a low molecular weight of less than 1.5 kDa and which mainly functions to arrest insoluble ferric iron from different environments, that are excreted under iron starvation by various organisms, including bacteria, fungi, and even some plants (6). Large numbers of bacterial genera are known to synthesize siderophores, such as *Pseudomonas, Azotobacter, Bacillus, Rhizobium,* and *Enterobacter* (7). Siderophores apart from their role in active transport of iron may act as growth antagonists by means of sequestering iron from the

environment, restricting the growth of pathogens. Siderophores, the metal-chelating agents produced by large number of bacteria under iron-destitute state, are mainly grouped into three types: hydroxamate, catecholate, and carboxylates (8).

Eichhornia crassipes or water hyacinth is invasive floating plants found in water bodies across the world. It has variety of negative impacts on the aquatic environments. *Eichhornia crassipes* is so called menace and nuisance, is considered as the world's worst aquatic plant but a highly productive aquatic weed that causes a serious hindrance to nation's development activities(9). Water hyacinth is considered a noxious weed species in more than 50 countries. The growth rate of water-hyacinth is among the highest of any known plant (10). The present investigation was planned to explore the potential of *Eichhornia crassipes* in a way that, its positive attributes outweigh the negative ones.

The present study have primary objectives such as isolation of strains from roots, leaves and also characterize them on the basis of morphological and biochemical. Moreover characterize strains on the basis of type of siderophore production. Isolated strains are a promising candidate for crop improvement under iron limiting condition due to its siderophore producing activities.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL AND ISOLATION OF ENDOPHYTES

Sample was collected from causeway road, Surat, Gujarat and authenticated by expert. After uprooting plants, leaf and root samples were washed thoroughly in running tap water to remove any adhering particle. Samples were surface sterilized by sequential immersion in 0.25% $HgCl_2$ for 1 minute, 95% ethanol for 30 seconds, then washing three times with sterile double distilled water. Sterilized roots and leaves were crushed using sterile glass rod and sap was streaked on nutrient agar (NA) plates and incubated for 2–3 days at 28°C ± 2°C. Colonies were selected on the basis of variation in morphology (color, size, and shape), purified, maintained on agar slants at 4°C and glycerol stocks at −20°C (11).

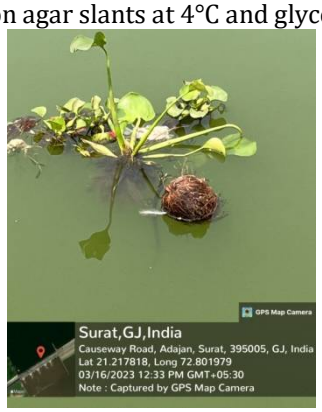

Figure 1: *Eichhornia crassipes*

SCREENING OF SIDEROPHORE PRODUCTION BY PLATE ASSAY:

Siderophore production by various putative PGPB isolates was determined following the universal assay of Schwyn and Neilands. In the assay, one can identify the siderophore producing bacteria through color change of the blue media. All the glassware were deferrated by washing with 6-M HCl overnight to eliminate the residual Fe contamination, then rinsed with double distilled water two to three times [12,13].

To prepare 1 Liter of CAS blue agar, 4 kinds of solution were needed: [13]

To prepare **solution 1**, i.e., the Fe-CAS indicator solution, 60.5 mg CAS (Sigma Aldrich, USA) was dissolved in 50 ml water and mixed with 10 ml iron (III) solution (1 mM FeCl3 •6H2O, 10 mM HCl). Under stirring condition, this solution was slowly added to 72.9 mg HDTMA dissolved in 40 ml water. The resultant dark blue liquid was autoclaved and cooled to 50^0 C.

To prepare buffer **solution 2**, 30.24 g PIPES was dissolved in 750 ml of salt solution containing 0.3 g KH2PO4, 0.5 g NaCl, and 1.0 g NH4Cl and a 50% (w/w) KOH solution was added to raise the pH of PIPES to 6.8. Solution 2 was autoclaved after adding 15 g of agar, and then cooled to 50^0 C.

To prepare **solution 3**, 100 ml of 15× KB medium (proteose peptone no. 3.2%, K_2HPO_4 0.115%, $MgSO_4 \cdot 7H_2O$ 0.15%, and glycerol 1.5%) was mixed with 70 ml of a solution containing 2 g glucose, 2 g mannitol, 439 mg $MgSO_4$ •$7H_2O$, 11 mg $CaCl_2$, 1.17 mg $MnSO_4$ •H_2O, 1.4 mg H_3BO_3, 0.04 mg $CuSO_4$ •$5H_2O$, 1.2 mg $ZnSO_4$ •$7H_2O$, and 1.0 mg Na_2MoO_4 •$2H_2O$. Solution 3 was autoclaved and cooled to 50^0 C.

To prepare **solution 4** was a 10% (w/v) casamino acid solution, the nitrogen source. 30 ml of 10% (w/v) casamino acid solution was filter-sterilized. All those four solutions were mixed and poured into petri dishes.

After solidifying, bacteria was spot inoculated on plate. Bacterial isolates inoculated on the CAS-blue agar were incubated for 4-5 days at 28±2°C. Following incubation, these plates were observed for production of yellow-orange halo zone around the spot which indicates siderophore production.

SIDEROPHORE PRODUCTION BY CAS LIQUID ASSAY:

In culture supernatant extracts, CAS solution was used to quantify siderophore activity (14). All strains were grown in a Nutrient Broth medium for 48 h on a rotary shaker (120 rpm) at 30 °C and then centrifuged for 15 min at 3000 rpm. 0.5 ml (9×108 cells ml−1) of the supernatant was mixed with 0.5 ml CAS solution and 10 µl shuttle solution (sulfosalicylic acid). After 2 h of incubation at room temperature, the absorbance of the mixture was read using UV/Vis spectrophotometer. Siderophores induce a color change in the CAS medium, which lowers the OD630 measurements, and siderophore production can be quantified using the following formula:

Siderophore unit (SU) = Ar − As /Ar × 100%

Where , Ar - the absorbance of the reference at 630 nm.

As - the absorbance of the sample (bacterial cultures) at 630 nm

DETERMINATION ON TYPE OF SIDEROPHORE:

The catecholate type of siderophore was determined by the Arnow's test [15]. In this assay,the culture supernatants obtained from quantitative assay were subjected to determine. 3 ml of the culture supernatant was then mixed with 0.3 ml of 5 N HCl solution, 1.5 ml of Arnow's reagent (10 g $NaNO_2$, 10 g $Na_2MoO_4.2H_2O$ dissolved in 50 ml distilled water) and 0.3 ml of 10 N NaOH. Then the mixture was incubated for 10 min to complete the reaction in which the presence or absence of pink colour was observed.

Csáky test was used for assay of hydroxamate siderophore production [15]. One milliliter of sample culture broth with 1 ml of H_2SO_4 (6 M) was mixed and autoclaved for 30 min at 121 °C. Upon cooling, 1 ml of sulfanilic acid (1% w/v) prepared in acetic acid 30% (v/v) with 0.5 ml iodine (1.3% w/v) was mixed and this mixture was incubated for 5 min at room temperature. Further, excess iodine was removed by adding 1 ml of trisodium arsenite (Na_3AsO_2) (2% w/v) prepared in water. Finally, 1 ml solution of α-naphthylamine (0.3% w/v) prepared in acetic acid 30% (v/v) was added to the solution resulting in change of color from orange to red.

RESULTS

ISOLATION OF ENDOPHYTES

A total ten morphological different bacterial endophytes were isolated from surface sterilized roots and leaves of selected aquatic weed plant. 05 isolates were from root (designated as ECR1, ECR2, ECR3, ECR4, and ECR5) and 05 isolates were from leaf (designated as ECL1, ECL2, ECL3, ECL4, and ECL5). Among them all the root strains showing positive for CAS plate assy. Out of total, 40% of the total isolates were gram positive and 60% were gram negative (Table 1). All bacterial strains exhibited variable colony and cell morphology.

Table 1: Gram's reaction results of isolated bacterial endophytes.

ISOLATES (ROOTS)	GRAM'S TEST	ISOLATES (LEAVES)	GRAM'S TEST
ECR1	+ve	ECL1	+ve
ECR2	-ve	ECL2	+ve
ECR3	-ve	ECL3	+ve
ECR4	+ve	ECL4	-ve
ECR5	-ve	ECL5	+ve

SCREENING OF SIDEROPHORE PRODUCTION BY QUALITATIVE AND QUANTITATIVE METHOD:

All the root bacterial strains were found positive for siderophore production and selected for quantitative estimation of siderophore production. Quantification of siderophore production in CAS liquid assay is shown n figure 2.

Figure 2: Siderophore production by root endophytic bacteria on CAS agar plate.

Percentage wise siderophore production by endphytic strains from plant is shown in table 2. Strains were producing siderophore in range of 30% to 56% (Table 2). Among them ECR3 prodced significantly higher quantities of siderophore of 55.660±0.785.

Table 2: Percentage siderophore production of bacterial endophytes.

ISOLATE	SIDEROPHORE PRODUCTION (CAS plate assay)	% SIDEROPHORE PRODUCTION (Siderophore unit) (CAS liquid assay)
ECL1	-	-
ECL2	-	-
ECL3	-	-
ECL4	-	-
ECL5	-	-
ECR1	+	34.303±0.771
ECR2	++	41.903±0.574
ECR3	+++	55.660±0.785
ECR4	+	35.903±1.470
ECR5	+	44.983±1.525

(+ Average, ++ Satisfactory, +++ Excellent)

Quantitative s production was determinated in a CAS liquid medium by a color change of CAS medium from blue to yellow which is shown in figure 3.

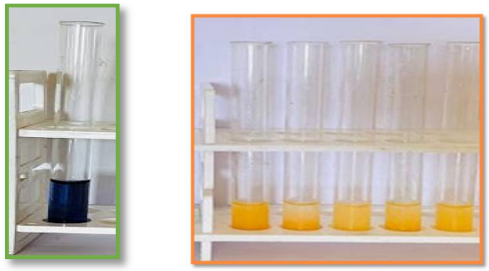

Figure 3: Siderophore production by endophytic bacteria in CAS liquid media

CHARACTERIZATION OF SIDEROPHORE:

Selected 05 bacterial isolates with significant siderophore production were characterized for type of siderophore produced (Figure 4) . Isolates, namely ECR3 & ECR5 were positive for carboxylate type of siderophore where as isolates namely ECR1, ECR2 & ECR4 were positive for hydroxamate type of siderophore. (Table 3)

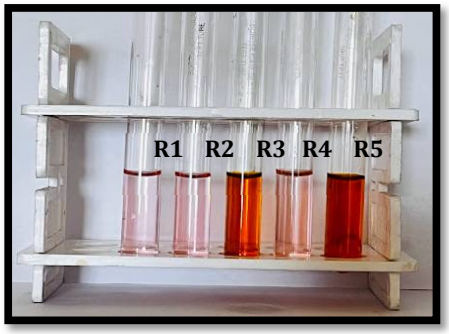

Figure 4:Characterization of siderophore production.

Table 3: Characterization of siderophore production by bacterial endophytes

ARNOW'S TEST (CATECHOLATE SIDEROPHORE)	CASKY'S TEST (HYDROXAMATE SIDEROPHORE)
ECR1, ECR2, ECR4	ECR3, ECR5

DISCUSSION

Endophytic bacteria do have several potential applications in various sectors of biotechnology including agriculture. One of the potency of bacterial endophytes in agriculture is to enhance the growth of agricultural crops. Microbial siderophore are subject of more interest because of chelating ferric iron, produced under low iron stress. Siderophores are produced by several microorganisms such as *Pseudomonas, Enterobacter* and *Escherichia coli* (16). The growing knowledge of bacterial endophytes with their favorable effects on plant growth has been recognised and can be a useful tools on the way to improve crop yield in addition to more sustainable and chemical free agriculture. Several studies on rhizobial as well as non-rhizobial endophytic bacterial genera during the last two decades have acknowledged their role in plant growth promotion(17).

In the present study, total of 10 bacterial endophytes isolated from roots and leaves of *Eichhornia crassipes,* and they were screened for siderophore production. Total five isolates from root showed orange colour halo zone on CAS agar plate. Production of siderophores is an important attribute of plant growth promoting endophytes and facilitates growth of plants under iron limiting conditions through iron sequestration (18). This media is very sensitive to variations in pH or FeCl3 concentrations. Schwyn and Neiland (12) developed a universal siderophore assay using chrome azurol S (CAS) and hexadecyl trimethyl ammonium bromide (HDTMA) as indicators. The CAS/ HDTMA complexes tightly with ferric iron to produce a blue color. When a strong iron chelator such as a siderophore removes iron from the dye complex, the color changes from blue to orange. There are many reports in literature on siderophore producing capacity of endophytes affirmed using CAS assay (19).

The production of siderophore was roughly estimated on the basis of size of halo formation on CAS agar. CAS agar method can only give rough idea and is not a perfect method for quantification of siderophore production. Hence, quantitative estimation of siderophore is done using liquid culture media and CAS reagent (20). In the present study 05 isolates of roots were selected for quantitative estimation. Amount of siderophore produced by all the 05 strains was checked. For control or absorbance reference (Ar) uninoculated broth and CAS reagent were kept. Concentration of siderophore production by bacterial strain varied from 34.303±0.771 to 55.660±0.785 SU. The maximum concentration of siderophore production was found in bacterial strain ECR3 with 55.660±0.785 SU. Similar results were found with 21, with concentration of siderophore production n range of 28.102±0.056 to 60.241±0.102 SU. Quantitatively also *P. aeruginosa* (KA19) produced maximum amount of siderophore 60.241±0.102 SU by spectrophotometer (traditional method) (21).

Very few studies report that rhizobia are good producers of siderophores 22. Endophytic strain *K. pseudosacchari* (LN) is being reported for the first time as an efficient siderophore producer. Although production of siderophore is a common phenomenon among PGPR present in rhizosphere, recent researches have also shown their production by endophytes residing in the plant tissues and role in plant growth promotion (23,24).

Siderophores produced by microorganisms are usually classified as catecholates, hydroxamates, carboxylates, and mixed type. A biochemical assays were used for determining siderophore type (15).

The catecholate type of siderophore was detected by arnow's test. Three isolates ECR1, ECR2 and ECR4 were positive for catecholate type of siderophore. Earlier studies also reported that several endophytic

bacteria could produce cateholate type of siderophore. Studies repoted that *Klebsiella pneumoniae* produced catecholate type of siderophore (15,25,26).
The hydroxamate type of siderophore was detected by casky's test. Only two of the isolate ECR3 and ECR5 produced hydroxamate type of siderophore. Studies reported that *Pseudomonas aeruginosa* produced hydroxamate type of siderophore by casky's test and FeCl 3 test (15,27,28).
The type of siderophore synthesized by bacteria depends on the amount and accessibility of nutrients and it may differ in culture rich conditions as compared to natural habitat. Previous studies have reported that the bacteria are capable of producing one or more types of siderophores, hydroxamate, and catecholate being the most common types (29,30) , but in contrary to this, our isolates produced both carboxylate and hydroxamate type of siderophores.

CONCLUSION

Siderophore production is very important trait involved in growth promotion as well as biocontrol of phytopathogens. A total of 10 endophytic bacteria isolated from roots and leaves of *Eichhornia crassipes* plant and were screened for siderophore production. All root bacterial isolates gave orange halo zone on CAS agar plate and were further selected for investigation for quantitative assay of CAS liquid assy. Upon quantitative analysis, 05 isolates produced siderophore units in range of 34% to 56% and subsequently characterized for the type of siderophore. Majority of them produced catecholate type of siderophore i.e. ECR1, ECR2, ECR4 and two of them produced hydroxamate type of siderophore i.e. ECR3 and ECR5. These ability to produce different types of siderophores with differences in structure and iron binding/ releasing capacities, enable these microbes to have multiple roles depending upon the habitats. The potential of these isolates for capability in enhancing plant growth can be examined and further plant growth promoting studies of this isolate can help to unravel the opportunities of this isolate for its use in bio-inoculation for sustainable agricultural practices.

REFERENCES

1. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett;278:1–9
2. Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR. (2016). Plant growth-promoting bacterial endophytes. Microbiol Res;183:92–9
3. Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P. (2016) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res;23:3984–99.
4. Maheshwari, R., Bhutani, N., & Suneja, P. (2019). Screening and characterization of siderophore producing endophytic bacteria from Cicer arietinum and Pisum sativum plants. *Journal of Applied Biology and Biotechnology*, *7*(5), 7-14.
5. Wittenwiler M. (2007).Mechanisms of iron mobilization by siderophores. Master Studies in Environmental Sciences Master, ETH Zürich.
6. Ahmed E, Holmström SJM. (2015) Siderophore production by microorganisms isolated from a Podzol soil profile. Geomicrobiol J; 32:397–411.
7. Kuzyk, S. B., Hughes, E., & Yurkov, V. (2021). Discovery of siderophore and metallophore production in the aerobic anoxygenic phototrophs. *Microorganisms*, *9*(5), 959.
8. Loaces, I., Ferrando, L., & Fernández Scavino, A. (2011). Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. *Microbial ecology*, *61*, 606-618.
9. Sari, N. H., Suteja, Rangappa, S. M., & Siengchin, S. (2023). A Review on Cellulose Fibers from Eichornia Crassipes: Synthesis, Modification, Properties and Their Composites. *Journal of Natural Fibers*, *20*(1), 2162179.
10. Arivendan, A., Jebas Thangiah, W. J., Das, R., Ahamad, D., & Chithra, G. K. (2023). Effect of water hyacinth (Eichhornia crassipes) plant into water bodies and its composite materials for commercial applications. *Proceedings of the Institution of Mechanical Engineers, Part C: Journal of Mechanical Engineering Science*, 09544062231166829.
11. Hung, P. Q., & Annapurna, K. (2004). Isolation and characterization of endophytic bacteria in soybean (Glycine sp.). *Omonrice*, *12*, 92-101.
12. Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical biochemistry*, *160*(1), 47-56.
13. Louden, B. C., Haarmann, D., & Lynne, A. M. (2011). Use of blue agar CAS assay for siderophore detection. *Journal of microbiology & biology education*, *12*(1), 51-53.
14. Nalli, Y., Singh, S., Gajjar, A., Mahizhaveni, B., Dusthackeer, V. N. A., & Shinde, P. B. (2023). Bacillibactin class siderophores produced by the endophyte Bacillus subtilis NPROOT3 as antimycobacterial agents. *Letters in Applied Microbiology*, *76*(2), ovac026.
15. Arora, N. K., & Verma, M. (2017). Modified microplate method for rapid and efficient estimation of siderophore produced by bacteria. *3 Biotech*, *7*(6), 381.

16. Rungin, S., Indananda, C., Suttiviriya, P., Kruasuwan, W., Jaemsaeng, R., & Thamchaipenet, A. (2012). Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a Thai jasmine rice plant (Oryza sativa L. cv. KDML105). *Antonie Van Leeuwenhoek*, *102*, 463-472.
17. Bharucha, U. D., Patel, K. C., & Trivedi, U. B. (2013). In vitro screening of isolates for its plant growth promoting activities from the rhizosphere of Alfalfa (Medicago sativa). *J Microbiol Biotech Res*, *3*(5), 79-88.
18. Nithyapriya, S., Lalitha, S., Sayyed, R. Z., Reddy, M. S., Dailin, D. J., El Enshasy, H. A., & Herlambang, S. (2021). Production, purification, and characterization of bacillibactin siderophore of Bacillus subtilis and its application for improvement in plant growth and oil content in sesame. *Sustainability*, *13*(10), 5394.
19. Ghazy, N., & El-Nahrawy, S. (2021). Siderophore production by Bacillus subtilis MF497446 and Pseudomonas koreensis MG209738 and their efficacy in controlling Cephalosporium maydis in maize plant. *Archives of microbiology*, *203*, 1195-1209.
20. Mrugesh, A. M. I. N. (2022). Characterization of siderophore produced by Pseudomonas sp. MT and its antagonist activity against Fusarium oxysporum f. sp. cubense and F. oxysporium f. sp. ciceris. *Notulae Scientia Biologicae*, *14*(4), 11298-11298.
21. Zhang, S., Deng, Z., Borham, A., Ma, Y., Wang, Y., Hu, J., & Bohu, T. (2023). Significance of Soil Siderophore-Producing Bacteria in Evaluation and Elevation of Crop Yield. *Horticulturae*, *9*(3), 370.
22. Chowdappa, S., Jagannath, S., Konappa, N., Udayashankar, A. C., & Jogaiah, S. (2020). Detection and characterization of antibacterial siderophores secreted by endophytic fungi from Cymbidium aloifolium. *Biomolecules*, *10*(10), 1412.
23. Abo-Zaid, G. A., Soliman, N. A. M., Abdullah, A. S., El-Sharouny, E. E., Matar, S. M., & Sabry, S. A. F. (2020). Maximization of siderophores production from biocontrol agents, Pseudomonas aeruginosa f2 and Pseudomonas fluorescens JY3 using batch and exponential fed-batch fermentation. *Processes*, *8*(4), 455.
24. Anupama, N.B.; Jogaiah, S.; Ito, S.; Amruthesh, K.N.; Tran, L.-S.P. (2015) Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. Plant Sci., 231, 62–73.
25. Pluháček, T., Lemr, K., Ghosh, D., Milde, D., Novák, J., & Havlíček, V. (2016). Characterization of microbial siderophores by mass spectrometry. *Mass spectrometry reviews*, *35*(1), 35-47.
26. Sullivan, J. T., Jeffery, E. F., Shannon, J. D., & Ramakrishnan, G. (2006). Characterization of the siderophore of Francisella tularensis and role of fslA in siderophore production. *Journal of bacteriology*, *188*(11), 3785-3795.
27. Costa, J. M., & Loper, J. E. (1994). Characterization of siderophore production by the biological control agent Enterobacter cloacae. *MPMI-Molecular Plant Microbe Interactions*, *7*(4), 440-448.
28. Shah, S., Karkhanis, V., & Desai, A. (1992). Isolation and characterization of siderophore, with antimicrobial activity, from Azospirillum lipoferum M. *Current Microbiology*, *25*, 347-351.
29. Farokh RZ, Sachdev D, Pour NK, Engineer A, Pardesi KR, Zinjarde S. (2011). Characterization of plant-growth-promoting traits of Acinetobacter species isolated from rhizosphere of Pennisetum glaucum. J Microbiol Biotechnol 21:556–66.
30. Kumar P, Thakur S, Dhingra GK, Singh A, Pal MK, Harshvardhan K (2011). Inoculation of siderophore producing rhizobacteria and their consortium for growth enhancement of wheat plant. Biocatal Agric Biotechnol 15:264–9.