Annals of Plant and Soil Research 26(2): 545-548 (2024)
https://doi.org/10.47815/apsr.2024.10397
Short Communication

Comparative analysis of siderophore production in marine and terrestrial pseudomonas species

H. PRASAD[1], P. UCHGOANKAR[2], S. SINGH[2*] AND D. DAS GUPTA[2]

[1]D.Y. Patil International School, D.Y. Patil University, Navi Mumbai, India
[2]School of Biotechnology and Bioinformatics, D.Y. Patil Deemed to be University, Navi Mumbai, India

Received, December, 2023; Revised accepted, May, 2024

Iron is one of the essential trace element for most organisms. Bacteria often obtain this nutrient via secreting low molecular weight biomolecules namely siderophores, which are secondary metabolites that take up iron from the environment and transfer it to cells through certain receptors (Kramer et al 2020). These siderophores secreted in response to iron starvation, help microbes to acquire iron from insoluble forms by mineralization and sequestration (Kannahi and Senbagam 2014). Additionally, quorum sensing, nutrient-stress and different environmental factors *viz.* carbon, nitrogen, pH, temperature and salt also influence siderophore production (Shen et al 2020). Microorganisms adapted to such environmental stress conditions synthesize siderophore-interacting proteins (SIPs) that promote the reduction of Fe^{+3} to Fe^{+2} allowing time and site specific delivery of iron, making it available to the cells (Rokolhuii and Srivastava 2014, Liu etal 2022). Siderophores have applications in weathering soil minerals and soil formation, enhancing plant growth as Plant Growth Promotion (PGP), biocontrol of pathogens, nuclear fuel processing, bioremediation of pollutants, recycling of iron in the ocean, bio-bleaching of pulps and as biosensors for iron detection (Anjali *et al.* 2015, Singh *et al.* 2022). The current system of employing the siderophore-drug complex represents a new and innovative therapeutic strategy against multidrug resistant (MDR) bacteria (Khasheii et al 2021). Previous research revealed that siderophores are produced by diverse terrestrial microbes living in various habitats. However marine siderophore producing bacteria have remained less explored (Sandy and Butler 2009). Hence, the current study focuses on siderophore production in terrestrial and marine isolates of *P. aeruginosa* to determine the factor that enhances the siderophore production, the effect of glycerol concentration and effect of time of incubation was also studied in both the isolates.

Two strains of *Pseudomonas aeruginosa* namely marine isolate MGPB31 (Ac No: MF511820) and terrestrial strain DKH3 (MCC Accession Number 2092) were selected for study. The quantitative estimation of siderophore was done by CAS shuttle assay (Murugappan et al 2011). The amount of siderophore production and optical density estimation was determined by using a spectrophotometer. Both the strains of *P. aeruginosa* were initially analysed for siderophore production using iron-depleted chemically defined minimal medium (M9). This solution was autoclaved and supplemented with 30 ml 10% (m/v) deferrated casamino acids (contaminated iron was removed with 3% 8-hydroxyquinoline in chloroform), 2.0 g l^{-1} glucose, 1 ml 1 M $MgCl_2$ and 1 ml 100 mM $CaCl_2$. These solutions were prepared and sterilized separately. After incubation, cultures were centrifuged at 10,000 rpm for 15 min and the cell-free supernatant (CFS) was tested for siderophore production using CAS shuttle assay (Murugappan *et al.* 2011).

Siderophore content in the CFS was calculated as explained by Uchgaonkar et al 2018. The factor selected for the current study was varying concentration of glycerol. The M9 medium was modified with varying concentrations of glycerol ranging from 0.2% to 0.6%. Siderophore production was monitored after every 24h for a period of 96h using CAS shuttle assay and %SU was determined for both the marine and the terrestrial isolate. There is a significant variation for siderophore produced by the marine and terrestrial isolates, for instance marine isolates gave maximum siderophore production as 54%SU after 72h whereas terrestrial isolates gave 62%SU after 48h. The

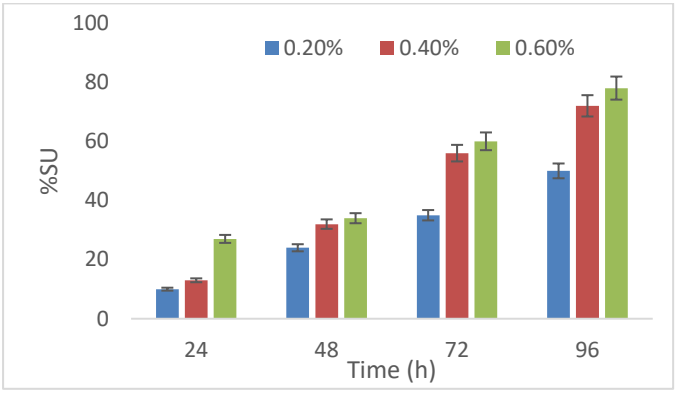
*Postal address: [2]School of Biotechnology and Bioinformatics, D. Y. Patil Deemed to be University, Navi Mumbai, Sector 15, CBD Belapur, Navi Mumbai, Maharashtra, 410210, India, *Corresponding author E mail: sunita.singh@dypatil.edu*

possible reason for this could be the higher growth rate of terrestrial isolates. Presence of glucose in M9 medium stimulates higher growth rate that possibly leads to a high cell density for terrestrial isolates in 48h. At this high cell density, siderophore production is maximum as these organisms compete for the available nutrients in the medium. A similar situation is observed for marine isolates but after 72h due to an extended lag phase as compared to their terrestrial counterparts. It has been reported that most of the bacterial isolates produce siderophores at an early log phase to meet their iron demand for metabolic processes like nucleic acid synthesis and respiration (Sinha et al 2019). The possible reason for this could be the dilute nature of the marine environment that presents many challeneges to bacteria in their quest to obtain iron required for growth. This results in delayed lag phase as compared to that of terrestrial isolates that exhibit a shorter lag phase (Sandy and Butler 2009).

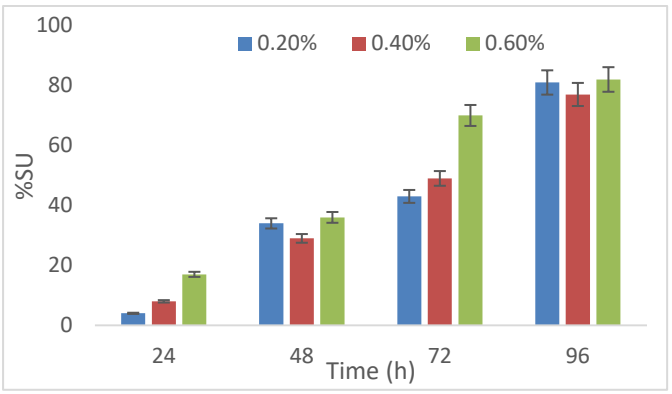
The results for effect of glycerol on siderophore production in marine and terrestrial isolate has been depicted in Fig. 1a. As depicted in the graph, the amount of siderophore produced in the marine strains gradually increases between the time period of 24 hours to 96 hours, for each concentration of glycerol. At a glycerol concentration of 0.2%, the amount of siderophore produced at 24 hours is at its lowest value (10%SU). As time proceeds, the siderophore production is maximum after 96h (70%SU). This trend is maintained throughout all the concentrations of glycerol, indicating a peak at the 96h and the lowest value at the 24h. The All strains of *P. aeruginosa* were inoculated in the modifiedM9 media with a 0.2%, 0.4% and 0.6% glycerol concentration. Incubation temperature was kept constant.

Fig. 1b analyses siderophore production in the presence of varying concentrations of glycerol for the terrestrial isolate. The amount siderophore produced in the terrestrial strains gradually decreases between the time period of 24 to 96 hours, in each concentration. At a glycerol concentration of 0.6%, the amount of siderophore produced at 24 hours is at its highest value (50%SU). As time proceeds, the amount of siderophore goes on decreasing recording minimum siderophore production (4%SU). This trend is maintained throughout all the concentrations of glycerol, indicating a peak at the 24-hour mark and the lowest value at the 96-hours mark. The graph also depicts a steady decrease in the siderophore units at each time interval as the concentration of glycerol increases from 0.2% to 0.6%.

Fig 1a also depicts a steady increase in siderophore units as the concentration of glycerol increases.



a



b

Fig. 1. Effect of Glycerol concentration on Siderophore production, in % siderophore units, over a time period of 96 hours in Marine (a) and Terrestrial (b) *P. aeruginosa*.

In the presence of glycerol, the siderophore production in marine isolates was found to increase with time. Glycerol enhances the production of compounds like salicylic acid, which is a precursor for siderophore production as also in *Pseudomonas flourescens* (Duffy and Defago 1999). In the dilute marine environment, glycerol stimulates the production of siderophore. Conversely, for the terrestrial isolates, replacement by glycerol in a minimal medium resulted in lower siderophore production. Similar results by Vindeirinho et al

(2021) suggest that a dilute environment is more appropriate or inducive for higher siderophore production and this is a tightly regulated process wherein once a maximum siderophore concentration has reached in the culture medium, the bacterium is unable to produce more of such compounds.

This could be co-related possibly for the need of iron for cell growth and secondly the establishment of a quorum that senses the need of iron at a particular cell density, which induces the cell to switch to siderophore production pathway (McRose et al 2018). Another possible reason may be that the low growth rate with the minimally available nutrients provides a perfect environment that stimulates the production of siderophores that benefit them in competing with the available resources in the medium. Nutrient availability can also contribute to siderophore production by affecting bacterial biofilm formation Donghoon et al 2019). The bacteria may have produced more siderophore in response to nutrient-stress or a decline in cell density. Overtime, the formation of biofilms may have been a likely cause for the increase in siderophore units. Therefore, when the marine strains form biofilms with a low optical density (O.D) and high siderophore production, they face low competition between bacterial cells which increases siderophore production (Eickhoff and Bassler 2020).

A one way ANOVA for these results indicate that for the terrestrial as well as marine isolates, the time of incubation has a strong positive correlation with the amount of siderophore produced with a significance factor of less than 0.05. Similar test for siderophore production against the glycerol concentration shows a significance factor of greater than 0.05 for both marine and terrestrial strains which indicates that glycerol concentration has no direct effect on the amount of siderophore produced at least among these two strains in the present study. As depicted from the results of the ANOVA test, with a significance factor of less than 0.05, it can be suggested that in the present culturing conditions time has a strong positive correlation with the amount of siderophore produced in both the strains. Sarvepalli et al (2023) along with Sairekha and Srividya (2016) have reported siderophore production in bacteria in the range of 24-120h of incubation. This could be due to the extended lag phase for marine isolates as compared to that for terrestrial isolates.

With growing anthropogenic activities and urbanization, the exploration for environmental-sustainable and suitable organic non-recalcitrant iron chelators, with specific properties to be used, as iron fertilizers, has become the need of the hour. The siderophore producing microbes can hence be exploited for use in agriculture, bioremediation methods for removal of toxic metals or also as drug conjugates (Dimpka 2016). This study provides useful insights on marine siderophores that have been less explored as compared to their terrestrial counterparts. The study also identifies the impact of glycerol on siderophore production as well as time as a significant factor in the commercial production of these siderophores by the marine isolate *P.aeruginosa.* Further, the effect of other media components as well as statistical based media optimization could help in significant increase in siderphore production for exploiting their use in the fields of agriculture, medicine and bioremediation.

REFERENCES

Anjali T, O.P. Verma and Manishi (2015) Isolation and PGPR characterization of cadmium resistant strains in cadmium contaminated soil. *Annals of Plant and Soil Research* **17** : 53-57.

Dimpka C. (2016) Microbial siderophores: Production, detection and application in agriculture and environment. *Endocyt Cell Res.* **27** (2): 7-16.

Donghoon A.K. (2019) Ph.D thesis “Biofilm-Mediated Regulation of Siderophore Production in *Pseudomonas aeruginosa”*

Duffy B and Defago G. (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas flourescens* biocontrol strains. Appl. *Envirol. Microbiol.* **65**(6): 2429– 2438.

Eickhoff M.J., Bassler B.L. (2020) *Vibrio fischeri* siderophore production drives competitive exclusion during dual-species growth. *Mol Microbiol*. **114**(2):244-261. https://doi.org/10.1111/mmi.14509

Kannahi M. and Senbagam N. (2014) Studies on siderophore production by microbial isolates obtained from rhizosphere soil and its antibacterial activity. *J of Chem and Pharm Res.* **6**(4): 1142–1145

Khasheii B, Mahmoodi P and Mohammadzadeh A. (2021) Siderophores: Importance in bacterial pathogenesis and applications in medicine and industry. *Microbiol Res. 250: 126790*. https://doi.org/10.1016/j. micres.2021.126790.

Kramer J, Özkaya Ö, Kümmerli R. (2020) Bacterial siderophores in community and host interactions. *Nat Rev Microbiol*. **18**(3):152-163. https://doi.org/10. 1038/s41579-019-0284-4

Liu L, Wang W., Wu S. and Gao H. (2022) Recent Advances in the Siderophore Biology of *Shewanella*. Front. Microbiol. **13**:823758. https://doi.org/10.3389/fmicb. 2022.823758.

McRose D.L., Baars O., Seyedsayamdost M.R., Morel FMM. (2018) Quorum sensing and iron regulate a two-for-one siderophore gene cluster in *Vibrio harveyi*. *Proc Natl Acad Sci USA.* 115(29):7581-7586. https://doi.org/10.1073/pnas.1805791115

Murugappan R.M., Aravinth A., Karthikeyan M. (2011) Chemical and structural characterization of hydroxamate siderophore produced by marine *Vibrio harveyi*, JIMB. **38** (2): 265 – 273.

Rokolhuii K. and Srivastava A.K. (2014) Substrate dynamics: developments and issues. *Annals of Plant and Soil Research* **16** (1): 1-8

Sairekha B. and Srividya S. (2016) Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli. *Agriculture and Natural Resources*, (50): **4**: 250–256, 2016.

Sandy M., Butler A. (2009). Microbial iron acquisition: marine and terrestrial siderophores. Chem Rev. (10):4580-95. https://doi.org/10.1021/cr9002787.

Sarvepalli M, Velidandi A, Korrapati N. (2023) Optimization of Siderophore Production in Three Marine Bacterial Isolates along with Their Heavy-Metal Chelation and Seed Germination Potential Determination. *Microorganisms*. **11**(12):2873. https://doi.org/10.3390/ microorganisms11122873

Sheng MM, Jia HK, Zhang GY, Zeng LN, Zhang TT, Long YH, Lan J, Hu ZQ, Zeng Z, Wang B, Liu HM. (2020) Siderophore Production by Rhizosphere Biological Control Bacteria *Brevibacillus brevis* GZDF3 of *Pinellia ternata* and Its Antifungal Effects on *Candida albicans*. *J. Microbiol Biotechnol.* **30**(5):689-699. https://doi.org/10.4014/jmb.1910.10066

Singh P., Chauhan P.K., Upadhyay S.K., Singh R.K., Dwivedi P, Wang J., Jain D. and Jiang M. (2022) Mechanistic Insights and Potential Use of Siderophores Producing Microbes in Rhizosphere for Mitigation of Stress in Plants Grown in Degraded Land. *Front. Microbiol.* **13**:898979. . https://doi.org/ 10.3389/fmicb. 2022.898979

Sinha A, Venkateshwaran B, Tripathy S, Sarkar A and Prabhakaran S. (2019) Effects of growth conditions on siderophore producing bacteria and siderophore production from Indian Ocean sector of Southern Ocean. *J Basic Microbiol*. 1–13. https://doi.org/10.1002/jobm.201800537

Uchgaonkar P., Padmadas N., Singh S. and Dasgupta D. (2018) Screening and identification of siderophore producing marine bacteria. *Global Journal of Bio-Science and Biotechnology*. **7**(3): 457 – 461.

Vindeirinho J.M., Soares H.M. and Soares E.V. (2021) Modulation of Siderophore Production by *Pseudomonas fluorescens* Through the Manipulation of the Culture Medium Composition. *Appl Biochem Biotechnol. **193**: 607–618.* https:// doi.org/10. 1007/s12010-020-03349-z

*