Applied Biological Research 26(1): 84-92; (2024)
DOI: 10.48165/abr.2024.26.01.12

EFFECT OF VARIOUS PHYSICOCHEMICAL PARAMETERS ON SIDEROPHORE PRODUCTION BY MARINE *Pseudomonas aeruginosa* MGPB31

Priti Uchgaonkar, Sunita Singh* and Debjani Dasgupta

School of Biotechnology and Bioinformatics, D.Y. Patil Deemed to be University, Navi Mumbai – 400 614, Maharashtra (India)
*****e-mail: *sunita.singh@dypatil.edu*

(Received 31 October, 2023; accepted 28 January, 2024)

ABSTRACT

Siderophores are low molecular weight compounds secreted by bacteria that bind ferric iron with extremely high affinity. The siderophores help bacteria to meet their iron needs under stress conditions as these compounds help in the transport and storage of soluble form of iron in cells. In current study, we report the effect of various physicochemical parameters that influence siderophore production in marine *Pseudomonas aeruginosa* MGPB31. Siderophore production was assayed using Chrome Azurol S (CAS) shuttle assay. The maximum siderophore production (56% SU) was obtained in minimal M9 medium after 72 h incubation, followed by succinate medium (52% SU) and King's medium (51% SU). The M9 medium was then modified by replacing its carbon, nitrogen and phosphate source. Enhanced siderophore production was observed in modified medium comprising of glycerol (70% SU), tri ammonium citrate (67% SU) and KH_2PO_4 (64% SU). The medium supplemented with iron exhibited a significant reduction in siderophore production. The effect of physical parameters like temperature, pH and agitation on siderophore production revealed that the isolate exhibited maximum siderophore production under static conditions in modified M9 medium devoid of iron at 25 ± 2°C and neutral pH. The enhanced siderophore production obtained by marine *P. aeruginosa* MGPB31 indicates its further applicability in fields of industry agriculture and environment.

Keywords: CAS, glycerol, iron, agitation, MM9 medium, siderophore

INTRODUCTION

Iron (Fe) is an important micronutrient for marine habitants, however due to its low solubility in seawater it exists in very low concentrations than the levels required for sustaining marine bacterial and phytoplankton growth (Park *et al.*, 2023). Under dilute nature of marine environment, the microbial communities have developed efficient iron acquisition strategies to meet their iron requirements (Manck *et al.*, 2022). One such strategy is the production of siderophores which are high affinity iron binding ligands secreted by bacteria to acquire their physiological demands of iron (Endicott *et al.,* 2020). The proteins located in bacterial cell surface recognize iron-siderophore complex with high specificity, and actively transport it into the cell. The iron from this complex is ultimately released in the cytoplasm rendering it available for various functions (Pattan *et al.,* 2017).

Fluorescent pseudomonads colonize various ecological niches and their adaptability is reflected by high diversity of their iron uptake systems. Majority of these fluorescent pseudomonads produce complex fluorescent peptide siderophores called pyoverdins or pseudobactins that act as efficient iron scavengers. Other less complex and lower affinity Fe^{3+} siderophores such as enantio/ pyochelin,

pseudomonine, nocardamin, etc. are synthesized by fluorescent and non-fluorescent *Pseudomonas* strains (Grosse *et al.,* 2023). Many strains of siderophore producing *Pseudomonas* co-exist with those of siderophore non-producers in both terrestrial and marine environments (Manak *et al.*, 2020; Kalyan *et al.*, 2022; Timofeeva *et al.*, 2022). Siderophore production is influenced by many factors like concentration of carbon, nitrogen and iron in their growth environment (Butaite *et al.*, 2018, Kalyan *et al.*, 2022). We previously observed enhanced siderophore production by *P. aeruginosa* MGPB31 in modified M9 (MM9) medium by applying a two stage statistical approach namely Plackett-Burman design and Response Surface Methodology (RSM); and also characterized these siderophores using FTIR and LC-MS analysis (Uchgaonkar *et al.,* personal communication). The present study focused on the characterization of *P. aeruginosa* MGPB31, isolated from the coastal area of Western India, by studying the effect of various physical parameters like pH, temperature and aeration on siderophore production. Since bacterial siderophore production is affected by the type of carbon and nitrogen source, therefore, the present study also attempted to find the most suitable growth medium; and asses the effect of various carbon and nitrogen sources, NaCl and iron on siderophore production by marine bacterium *Pseudomonas aeruginosa* strain MGPB31.

MATERIALS AND METHODS

Bacterial strain

The *Pseudomonas* isolate, used in present study, was isolated from the coastal area of Maharashtra India (Ganpatipule, latitude 17.1489° N, longitude 73.2727° E) and its identity confirmed as *Pseudomonas aeruginosa* strain MGPB31 by using MegaBlast (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*) and EzBioCloud-EzTaxon (*https://eztaxon-e.ezbiocloud.net*). The data of multiple sequence alignment was used for phylogenetic analysis and phylogenetic tree constructed by Neighbourhood Joining method with 1000 bootstrap. The siderophore production of this isolate was confirmed and quantified by CAS shuttle assay (Uchgaonkar *et al.*, 2018). For assessing the effects of various media and their components on siderophore production, 24 h old culture of *P. aeruginosa* MGPB31 (OD, 0.1 at 660 nm) was added @ 2%. The amount of siderophore produced was monitored after every 24 h for a period of 72 h by CAS shuttle assay (Uchgaonkar *et al.*, 2018). The amount of siderophore in CFS was calculated using the formula:

$$\% \text{ SU} = \frac{\text{Ar}-\text{As}}{\text{Ar}} \text{ x } 100$$

Where %SU is % Siderophore Units, and Ar and As are absorbance of reference and sample, respectively, at 630 nm

All the experiments were carried in triplicates and monitored for siderophore production.

Effect of different media on siderophore production

To assess the effect of culture media components on siderophore production, the isolate was grown on seven different media *viz*., sterile minimal medium (M9), glycerol, nutrient, King's, asparagine, succinate and marine broth (Sasirekha and Srividya, 2016). The 50 mL of each medium was separately inoculated with 2% saline culture of isolate (OD, 0.1 at 660 nm) and incubated at 25±2°C). All the experiments were conducted in triplicate and siderophore production from cell free supernatant was monitored by using CAS shuttle assay (Uchgaonkar *et al.,* 2018). The medium exhibiting higher siderophore production was further modified by changing its carbon and nitrogen sources.

Effect of carbon and nitrogen sources on siderophore production

The carbon and nitrogen sources and their utilization play indirect role in regulating the siderophore production (Murugappan *et al.,* 2012, Tailor and Joshi, 2012). Hence, the culture medium exhibiting enhanced siderophore production was further examined by replacing its carbon source by glycerol, starch, citric acid, malic acid and succinate @ 0.2%. Two other media included a combination of sugars like (a) succinate + glycerol, and (b) succinate + glucose (both in equal proportions) to examine

the effect on siderophore production. All the sugars were sterilised separately and added at a final concentration of 0.2%. The cell free supernatant was monitored for siderophore production as per Uchgaonkar *et al.* (2018).

To study the effect of nitrogen sources on siderophore production, the nitrogen in medium was replaced with different organic nitrogen sources like peptone, tryptone, yeast extract, urea and inorganic nitrogen sources like ammonium chloride, ammonium nitrate, tri-ammonium citrate (TAC), ammonium oxalate, ammonium sulphate and sodium nitrate (added @ 0.1%). All the experiments were conducted in triplicates and siderophore production was monitored by using CAS shuttle assay.

Effect of different combinations of phosphate

The source of phosphate (Na_2HPO_4: KH_2PO_4, 2:1) in M9 medium was modified to assess its effect on siderophore production by MGPB31. This combination was modified (based on content of phosphorus) with NaH_2PO_4: KH_2PO_4 (combination 1); KH_2PO_4: K_2HPO_4 (combination 2) and NaH_2PO_4: K_2HPO_4 (combination 3), all in 2:1 ratio. Media with different combinations of phosphate were inoculated in triplicates and assayed for siderophore production by CAS shuttle assay.

Effect of NaCl and iron on siderophore production

The M9 medium was supplemented externally with variable concentrations of NaCl (0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%), and siderophore production monitored by using CAS assay. In order to determine the threshold level of iron at which siderophore production is repressed, the isolate MGPB31 was grown in medium externally supplemented with iron (5, 10, 50, 100, 150, 200, 250 and 300 µg mL^{-1}) (Sarvepalli *et al* 2023). The siderophore production was assessed by CAS shuttle assay.

Effect of pH, temperature and agitation on siderophore production

The effect of pH on siderophore production was monitored by adjusting the pH of medium in the range of 6-8 at 25 ± 2°C under static conditions. To study the effect of temperature on siderophore production, M9 medium (pH 7.0) was inoculated with 1% culture and incubated under static conditions at different temperatures *viz*., 4, 25, 37, 42 and 50°C. To study the effect of agitation, the inoculated M9 medium (pH 7.0) was incubated at 25°C under shaker (75 rpm) and static conditions. Siderophore production was monitored at 24 h interval for 72 h by CAS shuttle assay (Uchgaonkar *et al.*, 2018). All the experiments were conducted in triplicate and assayed for siderophore production by CAS shuttle assay.

From above study, a modified medium comprising of optimum levels of components was prepared and checked for siderophore production using CAS shuttle assay. This modified medium was further optimised by using a two stage statistical approach namely Plackett-Burman (PB) design and response surface methodology (RSM) so as to further enhance the siderophore production (Uchgaonkar *et al.*, unpublished data).

Statistical analysis

All the experiments were conducted in a completely randomized design with each treatment replicated three times. The data was statistically analysed for the standard error and significance at $P<0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

MegaBlast, EzBioCloud-EzTaxon revealed the isolate as *Pseudomonas aeruginosa* isolate MGPB31. The multiple sequence alignment used for phylogenetic analysis and the phylogenetic tree constructed by Neighbourhood Joining method with 1000 bootstrap confirmed its identity. The genomic sequence was deposited in GenBank database under Accession No. MF511820). (*https://www.ncbi.nlm.nih.gov/nuccore/MF511820*) [Uchgaonkar *et al.* communicated]. The

development of appropriate medium and the assessment of the impact of various physicochemical parameters are essential to optimize siderophore production.

Effect of media on siderophore production

The siderophore production by isolate MGPB31 indicated maximum siderophore production in M9 medium (56% siderophore units SU), followed by succinate medium (52% SU) and King's medium (51% SU) [Fig. 1]. It is possible that the dilute nature of M9 medium resembles in its composition to environment leading to enhanced siderophore production in the isolates of marine origin (Murugappan *et al.*, 2012). Radzki *et al.* (2013) have reported the use of modified M9 medium comprising of glycerol as carbon source with different salts of copper, zinc and manganese for siderophore production in soil bacterium *Chryseobacterium*.

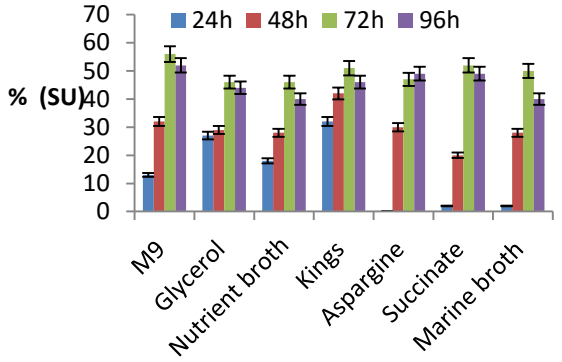

Fig. 1: Effect of various media on siderophore production by marine *Pseudomonas aeruginosa* MGPB31. The siderophore production was monitored every 24 h upto 96 h. All readings were taken in triplicates and the values are presented as mean ± SD

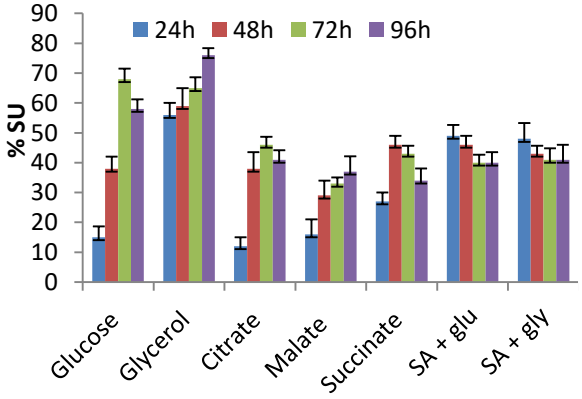

Fig. 2: Effect of carbon sources on siderophore production by marine *Pseudomonas aeruginosa* MGPB31 in M9 broth. The values presented are mean siderophore units ± SD

Effect of different C and N sources

Maximum siderophore production (70% SU) was observed in M9 broth with glycerol as carbon source after 96 h incubation (Fig. 2). Similar results were observed by Abo-Zaid *et al* (2020) where glycerol enhanced siderophore production in *Pseudomonas* isolates. This was consistent with the studies wherein siderophore production was enhanced in M9 medium where glucose was replaced with glycerol as carbon source in *Bacillus megaterium* and other *Bacillus* species (Santos *et al.*, 2014; Yu *et al.*, 2017). Glycerol enhances the production of compounds like salicylic acid which serve a precursor in siderophore production in *Pseudomonas fluorescens*. While sucrose enhanced siderophore production *in Bacillus licheniformis, B. subtilis*, and *Ochrobactrum grignonense* (Kalyan *et al.*, 2022). This revealed that in the isolated bacteria different carbon sources help in the regulation of siderophore production by adding specific moieties (Singh *et al*., 2020).

Most reports on siderophore production recommend the use of inorganic nitrogen sources rather than the organic sources because complex nitrogen sources are unable to boost cell growth and siderophore excretion (Sayyed *et al.*, 2010). There are enough reports wherein ammonium salts like ammonium chloride, ammonium nitrate or ammonium sulphate have been used in siderophore production (Sarvepalli *et al.*, 2023). The isolate MGPBS31 showed enhanced siderophore production (67% SU) when grown in medium containing inorganic nitrogen sources like tri-ammonium citrate (TAC) as compared to the organic nitrogen sources (Fig. 3). This could be due to the formation of additive citrate based siderophores which are chemically synthesised in natural sunlight conditions of sea water (Apelblat, 2014). In present study, isolate MGPB31 did not show enhanced siderophore production in presence of sodium, citrate alone. Possibly, the sodium citrate being a salt of strong base-weak acid would dissociate to give citrate ions which may associate with

available ferric irons dissolved in culture medium or flask thus making iron available to the organism. But at the same time, dissociation of sodium ions in medium would result in pH change making it alkaline resulting in reduced siderophore production in presence of sodium citrate. Thus siderophore production in presence of sodium citrate becomes slightly complex process in marine bacteria. However, when grown in presence of tri-ammonium citrate, the dissociation of citrate ions would be less. As a result, the formation of Fe-citrate as iron chelators to the organisms would be poor. This would impede iron utilization, thus compelling the microbe to enhance its siderophore production so as to mobilise iron in presence of TAC.

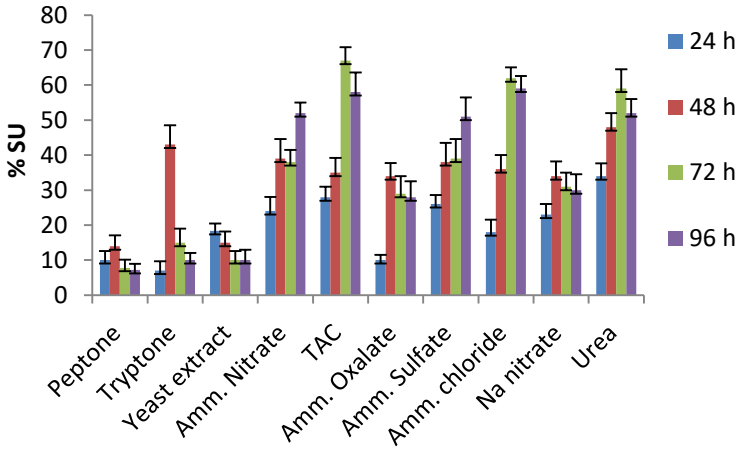

Fig. 3: Effect of different nitrogen sources on siderophore production by marine *Pseudomonas aeruginosa* MGPB31 in M9 broth with. Values presented are average siderophore units (of triplicates) ± S.D.

Effect of different combinations of phosphate

The study on the use of different combinations of phosphate salts for siderophore production by the isolate MGPB31 revealed that KH_2PO_4: K_2HPO_4 (2:1) exhibited slightly better siderophore production (64%SU) as compared to the control M9 (60% SU) and NaH_2PO_4: K_2HPO_4 (2:1) (45% SU) (Fig. 4). NaH_2PO_4: KH_2PO_4 (2:1) inhibited siderophore production. These results are in line with Cordero *et al.* (2012) who suggested that the interfering nature of phosphate buffer ($Na_2HPO_4.7H_2O$) chelates iron and leads to the discolouration of CAS dye. Further, Romanowski *et al.* (2011) demonstrated that the genes responsible for siderophore expression are suppressed without providing iron by creating conditions of local phosphate sufficiency.

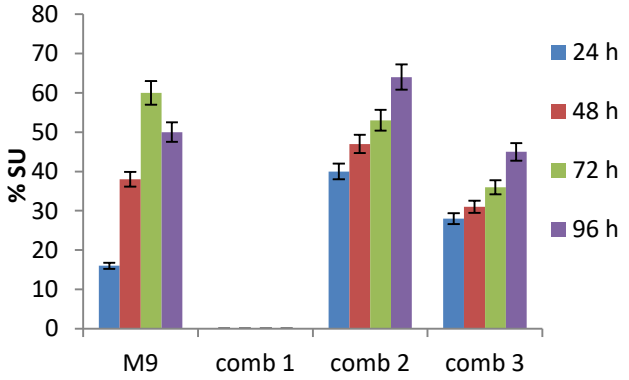

Fig. 4: Effect of different phosphate salts on siderophore production by marine *Pseudomonas aeruginosa* MGPB31 in M9 broth. Values presented are mean siderophore units ± SD; Comb 1 = NaH_2PO_4: KH_2PO_4 (2:1), Comb 2 = KH_2PO_4: K_2HPO_4 (2:1) and Comb 3 = NaH_2PO_4: K_2HPO_4 (2:1)

Effect of NaCl and iron on siderophore production

The concentration of NaCl plays a significant role in siderophore production and the isolate exhibited high salt (5%) tolerance (Uchgaonkar *et al.*, 2018). Maximum siderophore production (54% SU) was observed in M9 broth having 0.5% NaCl at 72 h (Fig. 5). With increase in NaCl concentration, the isolate exhibited more growth but yielded comparatively decreased siderophore production. Gaonkar and Bhosle (2013) have also reported decrease in siderophore production at NaCl concentration of $\geq$ 2% in case of *Bacillus* sp. Northover *et al.* (2021) found decreased zinc binding efficiency of siderophore functional groups with increase in salinity. However, the property of isolate to tolerate high

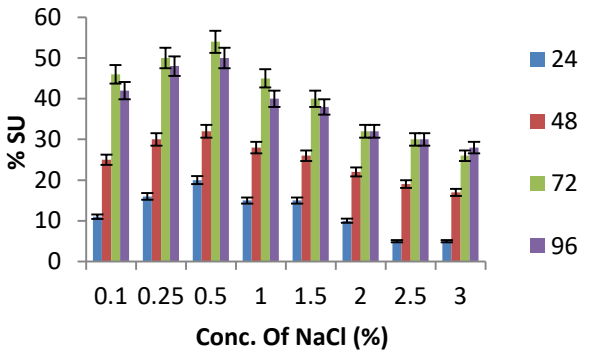

Fig. 5: Effect of different NaCl concentrations on siderophore production by marine *Pseudomonas aeruginosa* MGPB31 in M9 broth with Values presented are average siderophore units (of triplicates) ± S.D.

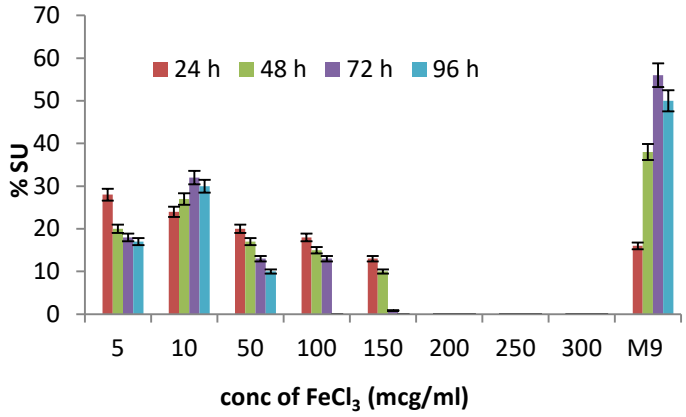

Fig. 6: Effect of various concentrations of iron on siderophore production by marine *Ps. aeruginosa* MGPB31 in M9 broth supplemented with Values presented are average siderophore units ± SD

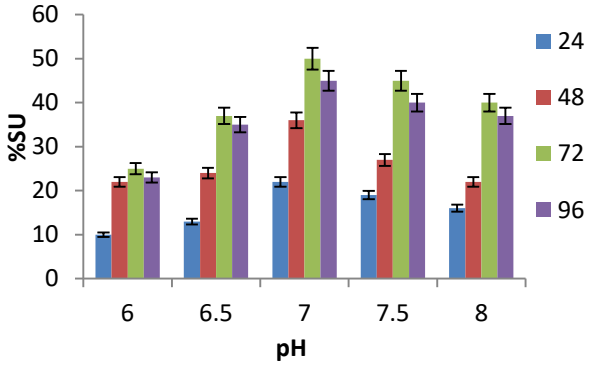

Fig. 7: Effect of pH on siderophore production by marine *Pseudomonas aeruginosa* MGPB31 in M9 broth at different pH. Values presented are mean siderophore units ± SD

NaCl concentration while still showing siderophore production could be exploited for use as salt tolerant plant growth promoter in alleviating soil salinity stress in plants (Uchgaonkar *et al.*, 2018).

The siderophore production by MGPB31 in M9 medium supplemented with iron indicated that the low levels of iron (5 - 150 µg mL^{-1}) stimulated siderophore production; however, the concentrations > 150 µg mL^{-1} inhibited siderophore production (Fig. 6). This may be attributed to the fact that siderophore transport system contains several gene products which are negatively regulated by iron binding protein for ferric uptake regulation (Sasirekha and Srividya, 2016). Thus, the study revealed that M9 medium is most suitable for siderophore production. This medium could be further modified by using glycerol as carbon source, tri-ammonium citrate as nitrogen source, and KH_2PO_4 as phosphate source (instead of Na_2HPO_4).

Effect of pH, temperature and agitation on siderophore production

pH plays an important role in the solubility of iron (Singh *et al.* 2020). Maximum siderophore production was noticed at pH 7.0 (Fig. 7) as it favoured the microbial growth. At this pH, iron in solution or medium exists in its insoluble form so is unavailable to bacteria (Jennifer *et al.*, 2015). Our results are in agreement with Srivastava *et al.* (2022) and Sarvepalli *et al.* (2023) who reported maximum siderophore production at neutral pH in case of *Pseudomonas* while acidic or alkaline conditions reduced siderophore production by inhibiting the bacterial growth.

The marine isolate MGPB31 could tolerate temperature in the range of 4-50°C; however, the siderophore production was less at very low or high temperatures (Fig. 8). Maximum siderophore production at 30°C has also been reported by Sarvepalli *et al.* (2023) in bacterial isolates of marine origin.

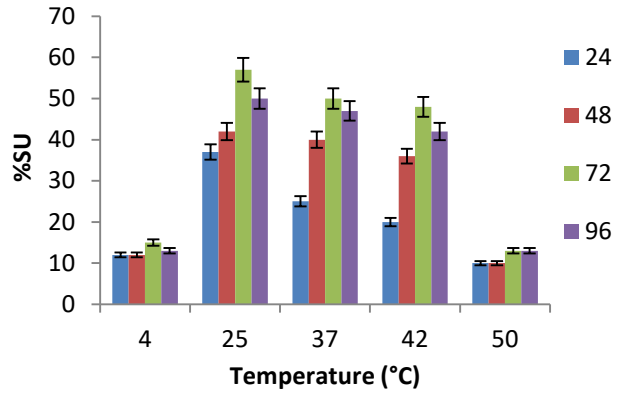

Fig. 8: Effect of temperature on siderophore production by marine *Pseudomonas aeruginosa* MGPB31 in M9 broth incubated at different temperatures. The values are mean siderophore units ± SD

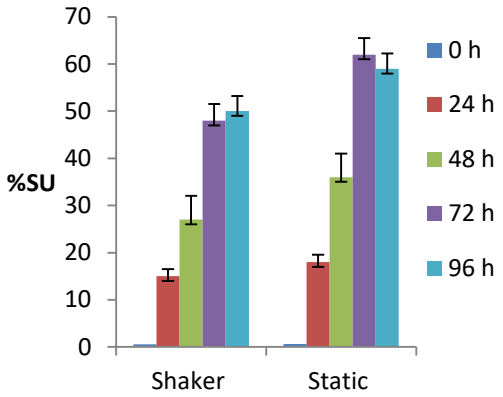

Fig. 9: Siderophore production by marine *P. aeruginosa* MGPB31 in M9 broth incubated at 25 ± 2°C under shaker and static conditions. The values are mean siderophore units ± SD

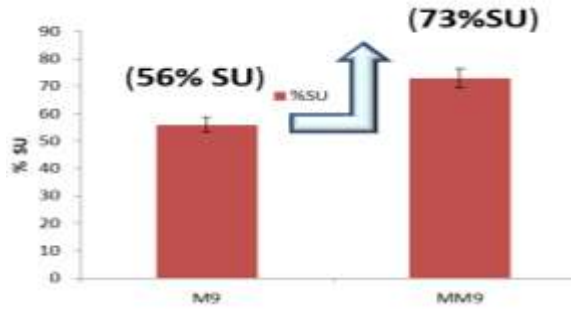

Fig. 10: Siderophore production by *Pseudomonas aeruginosa* MGPB31 in M9 medium and modified M9 medium

Siderophore production under shaker and static conditions for isolate MGPB31 indicated 14% increase in siderophore production under static conditions (Fig. 9). This is because under shaker conditions the growth rate is high so there is increased competition, thereby leading to a greater threat of "siderophore piracy" from the competitors. Hence under such conditions down regulation benefits the producer strain (Niehus *et al.*, 2017). This finding is significant as static conditions could reduce the overall costs involved in equipment and energy of the aeration and agitation process (Santos *et al.*, 2014). The results indicate that M9 broth exhibited better siderophore production at neutral pH, 25°C (room temperature) under static conditions. In this study the use of optimized physicochemical conditions boosted siderophore production from 56 % SU in M9 medium to 73% SU in modified M9 medium (Fig. 10).

Conclusion: The production of microbial metabolites has gained significant attention due to their diverse applications in many fields. In present study, the effect of various physicochemical parameters on siderophore production was studied in marine isolate MGPB31. The modified M9 medium enhanced siderophore production. This medium used glycerol as carbon source, tri-calcium citrate as nitrogen source and KH_2PO_4: K_2HPO_4 in 2:1 ratio. The modified M9 medium at neutral pH, room temperature and under static conditions further improved siderophore production as compared to the normal M9 medium by 30%. Further studies with statistical based approach for optimizing the modified M9 medium may help in identifying the key factors and the extent of parameter values that needs to be scaled up for enhancing large scale siderophore production.

REFERENCES

Abo-Zaid, G.A. Soliman, N.A., Abdullah, A.S., El-Sharouny, E.E., Matar, S.M. and 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**: 455. [*https://doi.org/10.3390/pr8040455*].

Apelblat, A. 2014. Formation of amides, citrate-based siderophores and other compounds. pp. 267-272. **In**: *Citric Acid* (1st edn.). Springer, New York, USA. [*DOI 10.1007/978-3-319-11233-6*].

Butaite, E., Kramer, J., Wyder, S. and Kummerli, R. 2018. Environmental determinants of pyoverdin production, exploitation and competition in natural *Pseudomonas* communities. *Environmental Microbiology*, **20**(10): 3629-3642.

Cordero, O., Ventouras, L., Delong, E. and Polz, M. 2012. Public good dynamics drive evolution of iron acquisition strategies in natural bacterioplankton populations. *Proceedings of the Natural Academy of Sciences*, **109**(49): 20059-20064.

Duffy, B. and Defago, G. 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas flourescens* biocontrol strains. *Journal of Applied and Environmental Microbiology*, **65**(6): 2429-2438.

Endicott, N., Rivera, G., Yang, J. and Wencewiz, T. 2020. Emergence of ferrichelatase activity in a siderophore-binding protein supports an iron shuttle in bacteria. A*CS Central Science*, **6(**4): 493-506.

Gaonkar, T. and Bhosle, S. 2013. Effect of metals on a siderophore producing bacterial isolate and its implications on microbial assisted bioremediation of metal contaminated soils. *Chemosphere*, **93**(9): 1835-1843.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedure for Agricultural Research* (2nd edn.). Willey, Hoboken, New Jersey, USA.

Grosse, C., Brandt, N., Van Antwerpen, P., Wintjens, R. and Matthijs S. 2023. Two new siderophores produced by *Pseudomonas* sp. NCIMB 10586: The anti-oomycete non-ribosomal peptide synthetase-dependent mupirochelin and the NRPS-independent triabactin. *Frontiers in Microbiol*ogy, **14**: 1143861. [*https://doi.org/10.3389/fmicb.2023.1143861*].

Guerinot, M., Meidl, E. and Plessner, O. 1990. Citrate as a siderophore in *Bradyrhizobium japonicum*. *Journal of Bacteriology*, **172**(6): 3298-3303.

Kalyan, V.K., Meena, S., Karthikeyan, S. and Jawahar, D. 2022. Isolation, screening, characterization, and optimization of bacterium isolated from calcareous soils for siderophore production. *Archives of Microbiol*ogy, **204**:721. [*http://dx.doi.org/10.1007/s00203-022-03322-w*].

Manck, L.E., Park, J., Tully, B.J., Poire, A.M., Bundy, R.M., Dupont, C.L. and Barbeau, K.A. 2022. Petrobactin, a siderophore produced by *Alteromonas*, mediates community iron acquisition in the global ocean. *International Society for Microbial Ecology*, **16**: 358-369.

Murugappan, R., Aravinth, A., Rajaroobia, R., Karthikeyan, M. and Alamelu, M. 2012. Optimization of MM9 constituents for enhancement of siderophoregenesis in marine *Pseudomonas putida* using response surface methodology. *Indian Journal of Microbiology*, **52**(3): 433-441.

Niehus, R., Picot, A., Oliveria, N., Mitri, S and Foster, K. 2017. The evolution of siderophore production as a competitive trait. *Evolution*, **71**(6): 1443-1455.

Northover, G.H.R., Mao, Y., Ahmed, H., Salvador Blasco, S., Vilar, R., Garcia-España, E., Weiss, D.J. 2021. Effect of salinity on the zinc(II) binding efficiency of siderophore functional groups and implications for salinity tolerance mechanisms in barley. *Scientific Report*, **11**: 16704. [*https://doi.org/10.1038/s41598-021-95736-7*].

Park, J., Durham, B.P., Key, R.S., Groussman, R.D., Bartolek, Z., Pinedo-Gonzalez, P., Hawco, N.J., John, S.G., Carlson, M.C.G., Lindell, D., Juranek, L.W., Ferrón, S., Ribalet, F., Armbrust, E.V., Ingalls, A.E. and Bundy, R.M. 2023. Siderophore production and utilization by marine bacteria in the North Pacific Ocean. *Limnology and Oceanography*, **68**: 1636-1653.

Pattan, J., Kajale, S. and Pattan, S. 2017. Isolation, production and optimization of siderophores from *Pseudomonas flourescens* NCIM 5096 and *Pseudomonas* from soil rhizosphere and marine water. *International Journal of Current Microbiology and Applied Science*, **6**(3): 919-928.

Radzki, W., Manero, G., Algar, E., Garcia, J., Villaraco, G. and Solano, B. 2013. Bacterial siderophores efficiently provide iron to iron starved tomato plants in hydroponics culture. *Antonie van Leeuwenhoek*, **104**(3): 321-330.

Romanowski, K., Zaborin, A., Fernandez, H., Poroyko, V., Valuckite, V., Gerdes, S., Liu, D., Zaborina, O. and Alverdy, J. 2011. Prevention of siderophore-mediated gut-derived sepsis due to *P. aeruginosa* can be achieved without iron provision by maintaining local phosphate abundance: role of pH. *BMC Microbiology*, **11**: 212. [*https://www.ncbi.nlm.nih.gov/pubmed/21943078*].

Santos, S., Neto, I., Machado, M., Soares, M. and Soares, V. 2014. Siderophore production by *Bacillus megatherium*: Effect of growth phase and cultural conditions. *Applied Biochemistry and Biotechnology*, **172**(1): 549-560.

Saraf, M., Sharma, S. and Thakkar, A. 2017. Production and optimization of siderophore from plant growth promoting bacteria. *Vidya*, **2**: 40-53.

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*].

Sasirekha, B. and Srividya, S. 2016. Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporiodes* causing diseases in chilli. *Agriculture and Natural Resources*, **50**: 250-256.

Sayyed, R., Gangurde, N., Patel, P. and Chincholkar, S. 2010. Siderophore production by *Alcaligenes faecalis* and its application for growth promotion in *Arachis hypogaea*. *Indian Journal of Biotechnology*, **9:** 302-307.

Singh, P., Khan A., Kumar, R., Kumar, R., Singh, V.K. and Srivastava, A. 2020. Recent developments in siderotyping: Procedure and application. *World Journal of Microbiology and Biotechnology*. **36**:178. [*https://doi.org/10.1007/s11274-020-02955-7*].

Srivastava, P., Sahgal, M., Sharma, K., Enshasy, H.A.E., Gafur, A., Alfarraj, S., Ansari, M.J. and Sayyed, R.Z. 2022. Optimization and identification of siderophores produced by *Pseudomonas monteilii* strain MN759447 and its antagonism toward fungi associated with mortality in *Dalbergia sissoo* plantation forests. *Frontiers in Plant Sciences*, **13**: 984522. [*https://doi.org/10.3389/fpls.2022.984522*].

Tailor, A.J. and Joshi, H.B. 2012. Characterization and optimization of siderophore production from *Pseudomonas fluorescens* strain isolated from sugarcane rhizosphere. *Journal of Environmental Research and Development*, **6**: 688-694.

Timofeeva, A.M., Galyamova, M.R. and Sedykh, S.E. 2022. Bacterial siderophores: Classification, biosynthesis, perspectives of use in agriculture. *Plants*, **11**: 3065. [*https://doi.org/10.3390/plants11223065*].

Venkat Kumar, S., Menon, S., Agarwal, H. and Gopalakrishnan, D. 2017. Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. *Resource-Efficient Technologies*, **3**(4): 434-439.

Uchgaonkar, P., Kudale S.; Singh S. and Dasgupta D. 2018a. Marine *Pseudomonas aeruginosa* MGPB31: A potential bioinoculum to alleviate salinity stress in spinach (*Spinacia oleracea)*. pp. 136-137. **In**: *Proceedings of the National Conference on Innovations in Food, Environment and Healthcare, Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 28-29 September, 2018, D. Y. Patil Deemed to be University, Navi Mumbai, India.

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.

Yu S., Teng C., Bai X., Liang J., Song T., Dong L., Jin Y. and Qu, J. 2017. Optimization of siderophore production by *Bacillus* sp. PZ-1 and its potential enhancement of phytoextration of PB from soil. *Journal of Microbiology and Biotechnology*, **27**: 1500. [*https://doi.org/10.4014/jmb.1705.05021*].