St. Joseph's Journal of Humanities and Science
ISSN: 2347 - 5331
http://sjctnc.edu.in/6107-2/

Production of Siderophore Using Bacterial Isolates From Rhizosphere of Paddy

- P. Poonguzhali*
- M. Saranya**

Abstract

The Siderophore productions of the bacterial isolates were investigated in the present study which is obtained from the rhizosphere of paddy. Among the total of thirty six isolates examined for the siderophore production using CAS (Chrome azurol sulfonate) agar plate assay, ten were producing siderophore activity. The positive bacterial isolates were belonging to the species of *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species and *E. coli*. The obtained positive isolates were further subjected to the production in the succinate medium with 1% inoculum. Among the various isolates, *Pseudomonas* species were found to exhibit high siderophore production of 92% and 83% respectively.

The best Siderophore producer was selected and processed with the optimization process. The parameters such as the pH, concentration of two different carbon sources (sucrose and succinic acid) along with the incubation time were examined for the optimization of siderophore. The highest siderophore production was observed at neutral pH. While with the sucrose and succinic acid as the carbon source, the high production was obtained to be the same with concentration of 1.5% whereas the effect of incubation period over the carbon sources revealed to be much decreasing when compared with maximal production. Thus, the potent organism from the rhizosphere region of paddy having the high siderophore activity could be subjected to industrial production for various applications.

Keywords: Siderophore, Chrome azurol sulfonate, Rhizosphere.

Introduction

Siderophores are defined as relatively low molecular weight ferric iron specific chelating agents synthesized by micro-organisms growing under low ionic stress. Chemically siderophores is an iron binding proteins ranging from 400-1500 Daltons in its molecular weight. They are produced by a wide range of micro-organisms under iron limiting conditions. Siderophore serve as the iron specific extracellular ligand to aid in the solubilisation and assimilation of iron. Microbial siderophore may stimulate plant growth directly by increasing the availability of iron in the soil surrounding the roots or indirectly by competitively inhibiting the growth of plant pathogens with less efficient iron uptake system.

*Assistant Professor, Department of Microbiology, St. Joseph's College of Arts and Science (Autonomous), Cuddalore-1. Email: poo_micro@rediffmail.com
**Department of Microbiology, St. Joseph's College of Arts and Science (Autonomous), Cuddalore-1.

Siderophore is used in medicine for iron and aluminium overload therapy, antibiotic for better targeting and suppurative therapy (Nagoba and Vedpathak, 2011). Siderophores have potential ability to resolve various environmental problems like heavy metal accumulation, rust removal, biofouling, dye degradation, sewage treatment and bioleaching etc. Siderophores are used in rust removal, because rust consists predominantly of Fe_2O_3 molecule with trivalent iron. Growth and any factors influencing either the growth or siderophore production by Plant growth promoting rizhobacteria (PGPR) would greatly influence the efficacy of that PGPR in plant growth promotion and disease suppression. The present study deals with the determination of the siderophore production by the bacterial isolates obtained from the rhizosphere region of the paddy field.

Materials and Methods

Isolation

Soil samples were collected from the rhizosphere soil of paddy field in a sterile container and further processed aseptically to obtain the bacterial isolates on nutrient agar using spread plate technique.

Screening for Siderophore Activity

The isolates were then screened for siderophore activity by plate assay. On the sterile modified Chrome azurol sulfonate (CAS) agar plate, wells were cut and 10µl broth cultures were added in each well of different isolates. Then the plates were incubated at 37°C for 24-48 hours. After incubation, colour change from blue to yellow orange halo around the growth was noted and the size of the holes around the growth was measured, which would indicate the total siderophore activity. The purified isolates were further subjected to the biochemical characterization for identification of organisms up to the genus level. The positive isolates were identified by staining, motility and biochemical test according to Bergey's manual of Systemic Bacteriology.

Quantitative Estimation

The estimation of siderophore was quantitatively done on succinate medium for the positive isolates using 1% inoculum. The flasks were then incubated for 24-48 hours at 28°C with constant shaking at 120 rpm on rotator shaker incubator. After incubation the fermented broth was centrifuged at 10,000rpm for 15 min. Supernatant was mixed with CAS solution. After 20 minutes of incubation. The absorbance of coloured solution was measured using spectrophotometer at 630nm. Similarly the assay was also carried with reference containing 0.5ml uninoculated succinate medium and 0.5ml CAS solution. The percentage of siderophore units was estimated as the proportion of CAS colour shifted (Meyer and Abdallah, 1978) using the formula,

$$\text{Siderophore units} = (Ar\text{-}As/Ar) \times 100$$

Where,

Ar = Absorbance of reference at 630 nm

As = Absorbance of sample at 630 nm

Effect of Different Parameters

The best producer was selected among all the positive isolates and subjected to optimization. Siderophore production was determined using various parameters which include pH levels (5, 6, 7, 8 & 9), Carbon source (ranging from 0.5%, 1%, 1.5%, 2% and 2.5%) succinic acid and sucrose and Incubation period (24 hrs & 48 hrs) respectively.

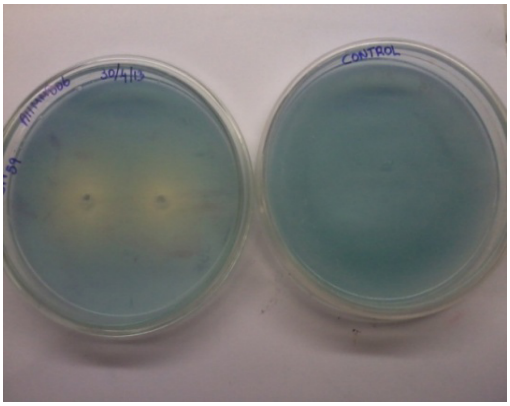
Result and Discussion

Siderophore production by bacteria is considered as important component of bacterial machinery for iron sufficiency and likely to be more important for the survival and growth in the competitive soil environment, which is usually deficient in soluble iron. The present study deals with the analysis of siderophore production by the siderophore producing bacterial isolate obtained from rhizosphere of paddy. Similarly, the earlier study of Misko and Germida (2002) suggest that the rhizosphere bacteria had an important role in the positively affects for the plant growth via different mechanisms.

Among the thirty six isolates obtained from ten samples, ten isolates (SPP50, SPP52, SPP53, SPP55 & SPP58- *Bacillus*; SPP51 & SPP61- *Pseudomonas*; SPP57- *Staphylococcus*; SPP63 & SPP69- *E. coli*) were found to produce siderophore activity, which was screened using siderophore plate assay. Isolates were screened on the CAS agar plates as described

by Schwyan and Neilands, (1987). The suggestion of modified CAS agar was made by Vagrali, (2009) and this modified CAS agar medium was employed in the present study producing yellowish orange haloes. It was indicating an iron chelator removing the iron from the blue CAS complex causing the colour change. In the earlier studies, the variation in colour changes in the CAS agar plate (yellow or orange, purple or purplish red) was observed in the production of siderophores of a differing nature by the variety of microorganisms.

Figure: 1 Screening for Siderophore by Plate Assay (Isolate SPP69)

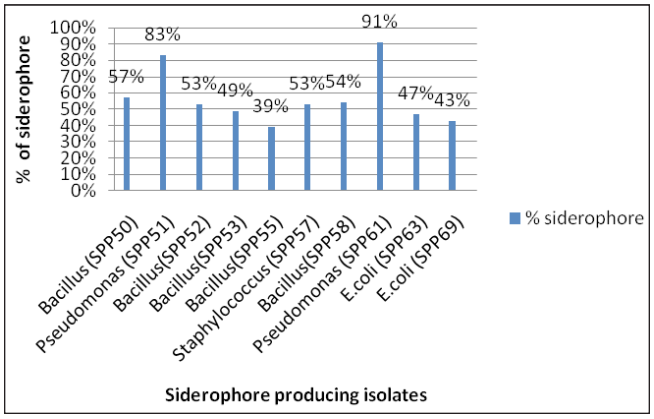


A B

Where, A is the CAS agar plate showing yellowishzone indicating the siderophore activity of the isolate SPP69 (*E.coli*) and B is the control plate.

Pal and Gokarn (2010) reported the siderophore activity exhibited by *Acinetobacter* and *E.coli* while *Bacillus subtilis* was reported by Hu and Xu, (2011). Most of the studies reported the *Pseudomonas* species from rhizosphere region (PGPR) in producing siderophore activity [Dhanya and Potty, (2007); Rachid and Ahmed, (2005)]. Similarly in our study, the ten positive isolates having the siderophore activity from rhizosphere region were identified as *Bacillus* species, *Pseudomonas* species, *Staphylococcus aureus* and *E.coli*. Five *Bacillus* species, two *Pseudomonas species*, two *E.coli* isolates and a *Staphylococcus* species were determined. In the quantitative estimation maximum percentage of siderophore production were obtained from two of the isolates which were belonging to the *Pseudomonas* species of about 91% and 83% respectively. Similar findings was also been reported by Sayyed and Chincholkar, (2010). Under iron deficiency conditions, microorganisms produce siderophores for Fe acquisition. Hence siderophores may serve as iron sources or as iron competitors for organisms, depending on the ability of the organisms to acquire iron from the stable iron siderophore complex.

Figure: 2 Percentage of Siderophore Production of Bacterial Isolates



Optimization of liquid culture conditions such as nutrients and other conditions such as pH and incubation period has profound impact on the quality and quantity of siderophore production. Correlating with the earlier reports of Hu and Xu,(2011) with increasing pH, siderophore production decreases that alkaline pH helps in excess solubilisation of iron, which increases their own content of the medium and results in decrease of siderophore production. Effect of the pH play a vital role in the siderophore production by the isolates, which was been clearly indicated from the data obtained. Moreover, the maximum siderophore production was detected to be high with the carbon source sucrose 83% when compared with the carbon source succinate. While the siderophore production using *Pseudomonas fluorescens* employed by Sayyed *et al.*, (2010) insisted on the sucrose and Mannitol as carbon source. The incubation time with combined carbon sources had its significant effect over the siderophore production, which showed much decrease on prolonged incubation than 24 hours. Thus it was suggested that siderophore production is substrate dependent and crucial factors such as substrate especially the carbon source and conditions like pH and time duration has an influence over improved siderophore production.

There is an enormous scope and multi sector application of microbial siderophores, agricultural and environmental sector for the sustainability of humans, animals and plants. But the siderophore research is not initiated in most of the microbiology research laboratories. So there is a need to insist on the organism

having elevated production, which may be helpful in the bioprocess technology for the industrial production of microbial siderophores.

References

1. Dhanya M.K and Potty V.P. 2007. Siderophore production by *Pseudomonas fluorescens* isolated from the rhizosphere of *Solenostemon rotundifolius*. *J. Root Crops. 32* (2):138-140.
2. Hu Q.P and Xu J.G. 2011. A simple double layered chrome azurol S agar plate assay to optimize the production of sidorophores by a potential biocontrol agent *Bacillus*. *African J. Microbio. Res*. 5(25): 4321-4327.
3. Meyer J.M. and Abdallah M.A.1978. The florescent pigment of *Pseudomonas flurorescence* biosynthesis, purification and physical chemical properties. *J.Gen. Microbiol*. 107:319-328.
4. Misko A.L and Germida J.J. 2002. Taxonomic and functional diversity of *Pseudomonads* isolated from the roots of field grown canola. *FEMS Microb*. 42:399-407.
5. Nagoba B and Vedpathak D. 2011. Medical applications of siderophores. *Eur. J.Gen.Med*. 8(3):229-235.
6. Pal R.B. and Gokarn.K.2010. Siderophores and pathogenecity of Microorganisms. *J.Biosci Tech*. 1(3):127-134.
7. Rachid D and Ahmed B. 2005. Effect of iron and growth inhibitors on siderophores production by *Pseudomonas fluorescens*. *African Journal of Biotech*. 4(7):697-702.
8. Sayyed R.Z and Chincholkar S.B. 2010. Growth and Siderophore production in *Alcaligenes faecalis* is regulated by metal irons. *Indian Journal of Microbial.* 50(2):179-182.
9. Sayyed R.Z., Gangurde N.S., Patel P.R, Joshi S.A and Chincholkar S.B. 2010. Siderophores production by *Alcaligenes faecalis* and *Arachis hypogaea*. *Indian Journal of Biotech*. 9:302-307.
10. Schwyn R and Neilands J.B. 1987. Universal chemical assay for detection and determination of siderophores. *Anal. Biochem*.160:47-56.
11. Vagarali M.A. 2009. Siderophore production by uropathogenic *Escherichia coli*. *India*