Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 1450-1452
Received: 04-05-2019
Accepted: 06-06-2019

Bagmare RR
Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India

Syed Ismail
Head, Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India

Ingole AJ
Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India

Bagmare PA
Post Graduate Institute of Post-Harvest Management, Killa, Roha, Maharashtra, India

Correspondence
Bagmare RR
Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India

Siderophore production by plant growth promoting microorganisms

Bagmare RR, Syed Ismail, Ingole AJ and Bagmare PA

Abstract
Under iron limiting conditions, many bacteria secrete ferric iron-specific ligands, generically termed siderophore, which aid in sequestering and transport of iron. We report here the production of siderophore by selected plant growth promoting microorganisms. Production of siderophore by plant growth promoting microorganisms were detected via the chrome azurol S assay, a general test for siderophore detection, which is independent of siderophore structure. Eight bacterial isolates having better plant growth promoting traits were evaluated for siderophore production in laboratory among the isolates *Pseudomonas flurescens* was noted to 75 per cent siderophore production followed by *Azospirillum lipoferum* (67 per cent). However, *Rhizobium phaseoli* found to have no colour change and no siderophore production in CAS agar test.

Keywords: Ligands, siderophore, chrome azurol S assay

Introduction
Rhizosphere is a dynamic environment which harbours diverse group of microorganisms. The bacteria which directly or indirectly stimulate the plant growth have been referred to as plant growth promoting rhizobacetria. PGPR promote growth of several annual crops by increasing uptake of nitrogen, iron (through siderophores), phosphorus, synthesis of phytohormones and by controlling plant diseases (Sayed *et al.* 2005). Iron is an essential trace element for living organisms. Because of its redox activities and ability to form co-ordination compounds with variety of ligands, it is a constituent of a large number of vital enzymes. Despite being most abundant elements in earth's crust, the availability of iron is limited by the very low solubility of Fe^{3+}(10 -17) predominant state of iron in aqueous, non-acidic, oxygenated environments and accumulates in mineral phases such as iron hydroxides as rust, hence cannot be utilized by organisms. In response microorganisms have developed a strategy for acquiring iron, which includes synthesis and utilization of siderophores.
A Siderophore (Greek for iron carrier) is a low molecular weight (500-1000 daltons), high affinity ferric iron chelating compound secreted by organisms. Siderophores scavenge iron from mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by energy dependent membrane transport mechanism and thus bind it and transport it to plants or bacterial cells. Siderophores which are wide spread among bacteria are of three types based on chemical nature of their co-ordination sites. Hydroxamate siderophore possesses N-hydroxylated amide bonds as co-ordination sites, catecholates co-ordinate iron with catecholate hydroxyl group and carboxylates co-ordinate iron with carboxyl and hydroxyl groups.

Material and method
Microbial strains and culture conditions
The laboratory stock cultures *Rhizobium phaseoli, Pseudomonas fluorescens, Pseudomonas striata, Bacillus subtilis, Bacillus polymyxa, Bacillus megaterium, Azotobacter chroococcum, Azospirillum lipoferum* and few others were procured from All India Network Project on Soil Biodiversity-Biofertilizers, VNMKV Parbhani and National Collection of Industrial Microorganisms **(**NCIM) Pune on the basis of their iron solubilizing ability in laboratory condition. The solubilization potential was evaluated both qualitatively and quantitatively under *in-vitro* condition as outlined in the following paragraphs:

Detection of siderophore
Siderophore production by plant growth promoting microorganisms was tested qualitatively by Chrome Azural S (CAS) liquid as well as plate assay. The strains were spread over CAS agar plate and incubated for 48 hrs at 28 ºC.

After incubation a thin layer of CAS reagent in 0.7% agar was spread on the bacterial growth and plates were again incubated for 24 hrs at 28 ºC, formation of yellow orange colour zone around the colonies in plate assay and colour changes from blue to orange in liquid assay, indicated the siderophore production (Schwyn and Neilands, 1987) [8]

Estimation of Siderophore

The quantitative estimation of siderophore produced by different plant growth promoting microorganisms was done by CAS-shuttle assay, in which both the strains were grown on CAS agar medium and incubated for 24-30 hrs at 28 ºC with constant shaking at 120 rpm on shaking incubator separately. During incubation, every 20 min 5 ml broths were centrifuged at 10,000 rpm at 4 ºC in cooling centrifuge for 10 minute and cell free supernatant was mixed with 0.5 ml CAS solution. The colour obtained was measured using the spectrophotometer at 630 nm with reference containing 0.5ml uninoculated succinate medium and 0.5 ml CAS solution. The percentage of siderophore unit was estimated as the proportion of CAS colour shifted using the formula: % Siderophore units = [(Ar - As)/Ar] x 100, where Ar is the absorbance at 630nm of reference (CAS assay solution+ uninoculated media) and as is the absorbance at 630nm of the sample (CAS assay solution + supernatant). (Payne, 1994) [6]

Results and Discussion

Screening for Siderophore producing ability

Siderophore production by different microbial isolates was confirmed by colour changes of CAS agar reagent from blue to orange. The colour change from blue to orange resulted by siderophoretic removal of Fe from dye.

Eight plant growth promoting microorganisms isolates tested, for their ability to produce siderophore under iron limiting condition, seven isolates were positive (Table no 1). It was obvious that all positive isolates produce siderophore on CAS assay. *Rhizobium phaseoli* do not shows growth on CAS agar plate. *Pseudomonas flurescens*, *Azotobacter chroococcum*, *Pseudomonas striata*, *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus megaterium*, *Azospirillum lipoferum* were positive on CAS agar test.

Quantitative CAS assay

In quantitative CAS assay, percent siderophore units were estimated as the proportion of CAS color shifted. *Pseudomonas flurescens* produced maximum amount of siderophore (75%), followed by *Azospirillum lipoferum* (67%), Bacillus subtilis (62%), Pseudomonas striata (58%) and Bacillus megaterium (44%). Same trend was observed in both qualitative and quantitative detection of siderophores produced by different plant growth promoting microorganisms.

Table 1: Siderophoregenesis by plant growth promoting organisms

Sr. No	Microbial inoculants	CAS Agar test	% Siderophore
1	*Rhizobium phaseoli*	-	0
2	*Azotobacter chroococcum*	+	35
3	*Pseudomonas striata*	+	58
4	*Bacillus subtilis*	+	62
5	*Bacillus polymyxa*	+	10
6	*Bacillus megaterium*	+	44
7	*Pseudomonas flurescens*	+	75
8	*Azospirillum lipoferum*	+	67

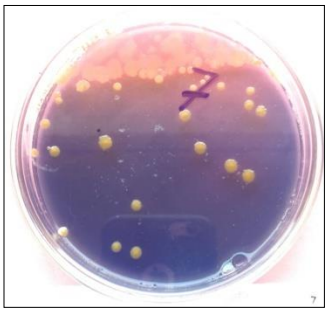

Fig 1: Formation of yellow-orange colonies on CAS agar plate

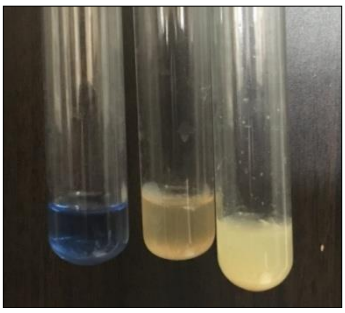

Fig 2: Colour chages of CAS reagent from blue to orange in qualitative CAS assay.

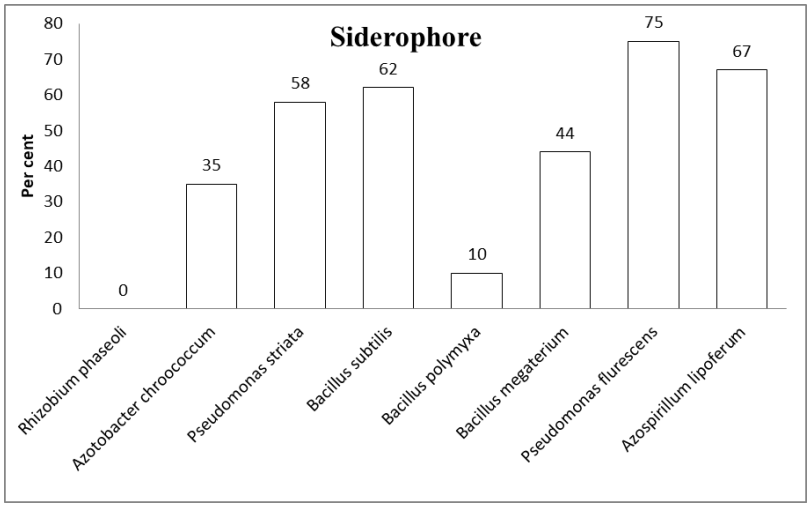

Fig 3: Siderophore production of different promising microorganisms on CAS agar plate.

Amount of siderophore produced by both the *Pseudomonas* sps. were estimated as percentage of siderophore units as the proportion of CAS color shifted. By liquid CAS assay, Pseudomonas fluorescens and *Pseudomonas aeruginosa* have shown the highest yields of siderophore i.e. 88% and 83% siderophore units respectively (Bholay *et al.* 2012) [1]

The two fluorescent *Pseudomonads*, *Pseudomonas fluorescens* NCIM 5096 and *P. putida* NCIM 2847 produced maximum yield of hydroxamate type of siderophore (87% and 83% units, respectively). (Sayyed *et al.* 2005) [7]. Pseudomonas fluorescens NCIM 5096 shows the high yellow zone formation on the CAS agar plate. Further similar finding was also reported by Gupta and Gopal (2008) [2] studied on different bacteria includes Bacillus coagulans, Bacillus sp., Bacillus *polymyxa, Brevibacillus brevis, Enterobacter sp., Pseudomonas sp., Pseudomonas fluorescens, Pseudomonas striata, Azospirillum brasilens, Enterobacter sp.* Among that *Pseudomonas fluorescens* (76%) produced maximum amount of siderophores followed by *Enterobacter sp., Pseudomonas sp., Enterobacter sp., Azospirillum brasilense and Brevibacillus brevis.* Same trend was observed in both qualitative and quantitative detection of siderophores produced by various PGPR isolate.

Mansoureh Sadat *et al.* (2012) [4] reported that *Pseudomonas fluorescens* forms a major constituent of Rhizobacteria that encourage the plant growth through their diverse mechanisms. In this investigation, 20 strains of *Pseudomonads* isolated from the rhizosphere soils of paddy areas in Malaysia and were screened for their plant growth promoting activity. All the 20 tested isolates of *Pseudomonads* were positive for the production of siderophores and HCN, while of the 20 antagonist bacteria strains, 15 strains (75%) showed positive for the production of plant growth-promoting hormone, IAA.

Conclusion

Among eight plant growth promoting microorganisms isolates tested for siderophore production. Only seven were found to produce more siderophore. Rhizobium phaseoli do not shows growth on CAS agar plate. Pseudomonas flurescens produced maximum amount of siderophore (75%) followed by *Azospirillum lipoferum* (67%).

Acknowledgement

We feel grateful to All India Network Project on Soil Biodiversity-Biofertilizers, VNMKV Parbhani and National Collection of Industrial Microorganisms (NCIM) Pune for providing the microbial strains for this study.

References

1. Bholay AD, Jadhav PU, Borkhataria BV, Dhalkari, MV. *Fluorescent pseudomonads* as plant growth promoting rhizobacteria and their siderophore genesis. Journal of Pharmacy and Biological Sciences. 2012; 3(1):27-32.
2. Gupta A, Gopal M. Siderophore production by plant growth promoting rhizobacteria. Indian Journal of Agricultural Research. 2008; 42(2):153-156.
3. Lemanceau P, Alabouvette C. Suppression of Fusarium wilts by fluorescent *Pseudomonads*: mechanisms and applications. Biocontrol Science and Technology. 1993; 3(3):219-234.
4. Mansoureh Sadat Sharifi Noori, Halimi Mohd Saud. Potential plant growth promoting activity of Pseudomonas species isolated from paddy soil in Malaysia as biocontrol agent. Journal of Plant Pathology and Microbiology. 2012; 3(2):1-4.
5. Parmar HY, Chakraborty H. Effect of siderophore on plant growth promotion. International Journal of Applied and Pure Science and Agriculture. 2016; 2(3):60-68.
6. Payne SM. Detection, isolation, and characterization of siderophores. Methods in Enzymology. 1994; 235:329.
7. Sayyed RZ, Badgujar MD, Sonawane HM, Mhaske MM, Chincholkar SB. Production of microbial iron chelators (Siderophores) by *Fluorescent pseudomonads*. Indian Journal of Biotechnology. 2005; 4:484-490.
8. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Analytical biochemistry. 1987; 160(1):47-56.