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Evaluation of Siderophore Production by Different Promising Microbial Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AI and SI designed the experiment. Author AI wrote the first draft and made statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Indian alkaline soils are deficient in iron due to high pH, high calcium carbonate, highly permeable coarse textured soils, and soils low in organic carbon content. This study is carried out to increase the iron use efficiency of soil by using promising microbial isolates. Production of siderophore by promising microbial isolates is a greatest invention which help in increasing uptake of iron. A short-term laboratory experiment was carried "To evaluate the siderophore productions by different promising microbial isolates using chrome azurol S assay technique". Ten microbial isolates were inoculated on plate as well as in broth medium. Plates were observed for orange halo formation around the bacterial growth. The size of orange halo around the colony of each microbial isolates was measured. The broth cultures inoculated with inoculum, incubated at 28 ± 2^0C for 24-72 hr with constant shaking at 120 rpm. After the incubation, the culture broths were centrifuged at 10,000

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rpm for 10 min. The cell free supernatants were subjected to quantitative estimation of siderophores by CAS-shuttle, absorbance was measured at 630 nm against a reference consisting of 0.5 ml of uninoculated broth and 0.5 ml of CAS reagent. Obtained results indicated that in both plate and broth assays medium inoculated with strain *Pseudomonas fluorescens* show significantly higher orange halo formation around the growth and maximum siderophore content in aliquot. Inoculation of this plant growth promoting microbial isolates will be the great achievement in replacing chemical fertilizers in agriculture.

Keywords: Siderophore production; microbial isolates; Chrome azurol S assay technique.

1. INTRODUCTION

"Iron, is a vital micronutrient needed for metabolism in all plants, due to its diverse role in the biosynthesis of chlorophyll, redox reactions and many physiological functions. The availability of iron to plants in neutral and alkaline soils are very less. Due to iron deficiency, the productivity of the crops is decreasing and this would lead to economic loss. Microbial siderophores are one of the major sources of iron to the plants. Siderophores are low molecular-weight organic compounds which are produced under iron-limiting conditions and change iron to available and absorbable form for crops and for microorganisms. Microorganisms have evolved a wide range of strategies enabling them to acquire iron from the environment, including reduction of Fe^{3+} to Fe^{2+} ions and the uptake of iron in the form of ferric siderophores (microbial iron chelates). They express a variety of low molecular weight, high affinity chelating agents that solubilize ferric iron in the environment and transport it into the cell and are generically known as siderophores" [1]. "Siderophores are commonly produced by aerobic and facultative anaerobic bacteria and fungi under iron limiting conditions. Research on siderophore-mediated iron transport has mainly focused on Gram-negative bacteria, although Gram-positive bacteria such as *Bacillus*, *Staphylococcus* and *Streptomyces* also possess siderophore-mediated iron transport systems [2]. To date nearly 500 siderophores are reported from selected microorganisms. In general, siderophores are classified as hydroxamates, catecholates, salicylates and carboxylates and more recently with new group polycarboxylates" [3,4]. "Variations are seen in siderophores structure from one species to another" [5]. "Siderophores and their substituted derivatives have varied applications in agricultural, environmental and medical sciences" [6].

"The mechanism of siderophore is to first bind with a ferric form of iron and form a complex of siderophore-iron that enters the cells through specific siderophore receptors present in the cell membrane. For gram-positive bacteria, transport of the siderophore-iron complex is carried out by the involvement of siderophore irrevocable proteins, permeases, and ATPases. Whereas, in the gram-negative bacteria the transport mechanism is quite different due to their complex membrane structure. Here, they transfer the siderophore-iron complex through a periplasmic binding protein and a cytoplasmic membrane protein corresponding to ABC transporter" [7]. "As soon as the complex enters the cytosol, the ferric iron (Fe^{3+}) gets reduced to a ferrous (Fe^{2+}) form which becomes free from the siderophore chelator complex. The released ferrous iron (Fe^{2+}) form is further utilized for plant and microbial metabolic processes" (Ahmed and Holmstrom, 2014). Though, the primary application of siderophore is to provide soluble iron to microbial communities for their growth. They also play various roles in fields such as Agriculture, Bioremediation, Biosensor, and Medicine. Hence, our study is focused on the selection of siderophore-producing bacteria collected from iron-enriched soil collected by All India Coordinated Research Project at VNMKV, Parbhani. This study enumerates the siderophore production and optimized culture condition in which the isolates produced a higher concentration of siderophores.

2. MATERIALS AND METHODS

2.1 Microbial Strains and Culture Conditions

The laboratory stock cultures (*Bacillus subtilis, Bacillus lecheniformis, Bacillus megaterium, Bacillus thuringiensis, Pseudomonas fluorescens, Pseudomonas striata, Trichoderma viride. Trichoderma herzenium, Azotobacter chroococcum and Azospirillum lipoferum*) were procured from All India Network Project on Soil Biodiversity-Biofertilizers, VNMKV Parbhani on the basis of their siderophore producing capacity

in laboratory condition. The siderophore production was evaluated both qualitatively and quantitatively under *in-vitro* condition as outlined in the following paragraphs:

2.1.1 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 28ºC. 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 28ºC, 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" [8].

2.1.2 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 28ºC 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 4ºC 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 630 nm of reference (CAS assay solution+ uninoculated media) and As is the absorbance at 630 nm of the sample (CAS assay solution + supernatant)" (Handerson and Payne, 1994). The data obtained was statistically analysed and appropriately interpreted as per the methods described in "Statistical Methods for Agricultural Workers" by Panse and Sukhatme.

3. RESULTS AND DISCUSSION

3.1 Siderophore Production

Siderophore production affected by plant growth promoting microbial strains was listed in Table 1 and Fig. 1. Siderophore production by various microbial strains was estimated by using CAS agar plate and broth medium in laboratory condition.

All ten strains tested was capable for siderophore production on CAS agar plate and broth medium. In that, strain *Pseudomonas fluorescens* shows significantly higher production of siderophore (52.83 %) (+++) inoculated in CAS agar plate and broth medium, which was followed by inoculation strain *Pseudomonas striata* (48.33%) (+++) and *Bacillus megaterium* (46.67%) (+++). These strains are superior over other strain and found at par with each other microbial isolates. Significantly lowest siderophore production was found inoculation of *Trichoderma herzenium* strain in CAS agar medium (15.00%) (+).

Table 1. Effect of different microbial agents on different plant growth promoting trades

Treatments	Siderophore production	
	%	
T_1 Uninoculated control	0.00	Nd
T_2 *Bacillus subtilis*	28.33	++
T_3 *Bacillus licheniformis*	31.00	++
T_4 *Bacillus megaterium*	46.67	+++
T_5 *Bacillus thurengenesis*	23.33	++
T_6 *Pseudomonas fluorocens*	52.83	+++
T_7 *Pseudomonas striata*	48.33	+++
T_8 *Trichoderma viride*	32.67	++
T_9 *Trichoderma herzenium*	15.00	+
T_{10} *Azotobactor chroococcum*	28.83	++
T_{11} *Azospirillum lipoferum*	23.33	++
S.Em.±	2.29	-
CD @ 5%	6.71	-
CV	13.19	-

The colour change of CAS from blue to orange to red may be due to siderophoral removal of Fe from the dye. Orange and yellow colorization around colony due to positive siderophore production. Also confirmed that *Pseudomonas fluorescens* (88%), *Bacillus spp.* (83%), *Trichoderama spp.* (62%) and *Azotobacter* (46%) isolates were siderophore producers reported by Ahemad and Kibret, 2014.

Significantly maximum siderophore production in CAS medium by *Pseudomonas fluorescens* (75%) followed by *Azospirillum lipoferum* (67%) and *Psudomonas striata* (58%) was brilliantly reported by Bagmare et al., [9]. Raval and Desai, [10], studied "the 86 bacterial strains belonging to *Pseudomonas*, *Enterobacter, Stenotrophomonas* and *Bacillus* from sunflower roots and associated

regions were tested for the siderophore production. In the CAS test, *Pseudomonas spp.* and *Enterobacter spp.* showed orange zone of decolourization on CAS agar plate whereas others produced light yellow colourization and were positive for siderophores" [11].

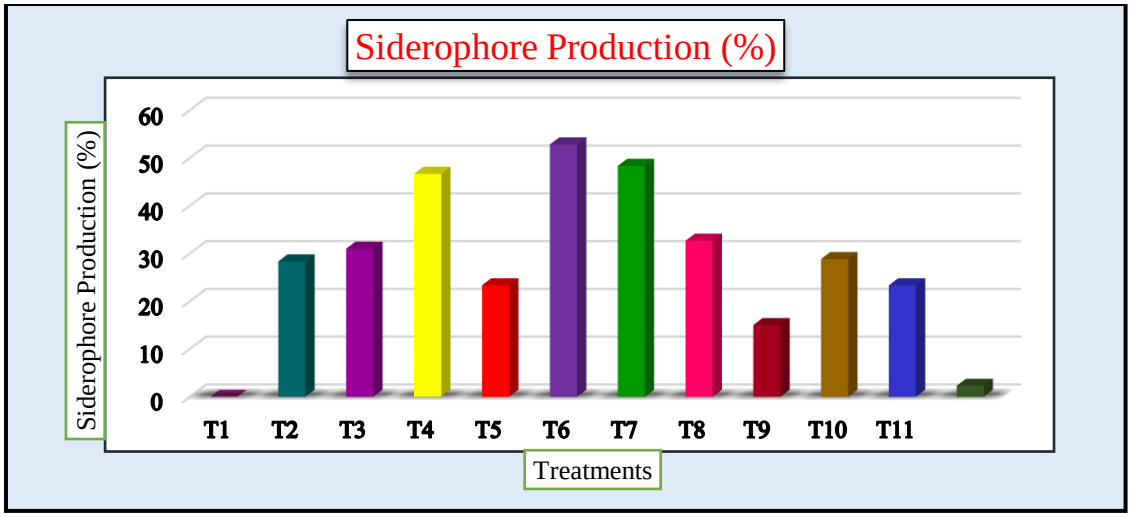
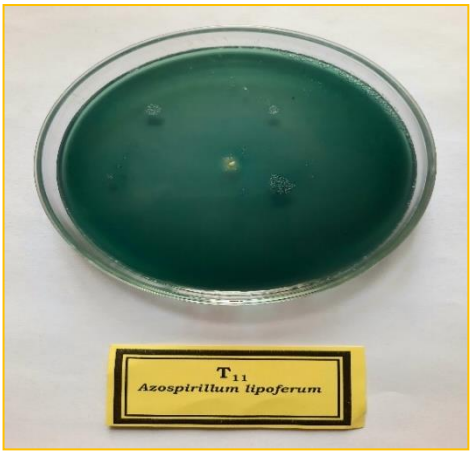
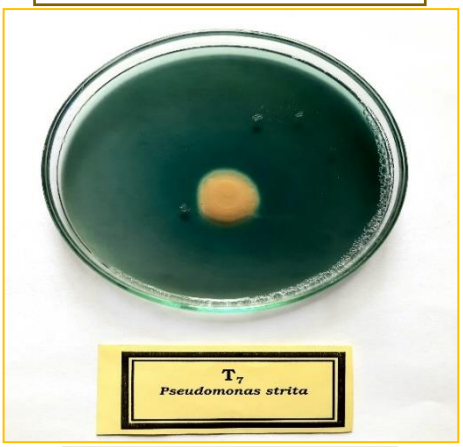


Fig. 1. Effect of different microbial agents on siderophore production



Azospirillum lipoferum

Bacillus megaterium



Pseudomonas straita

Pseudomonas flurocens

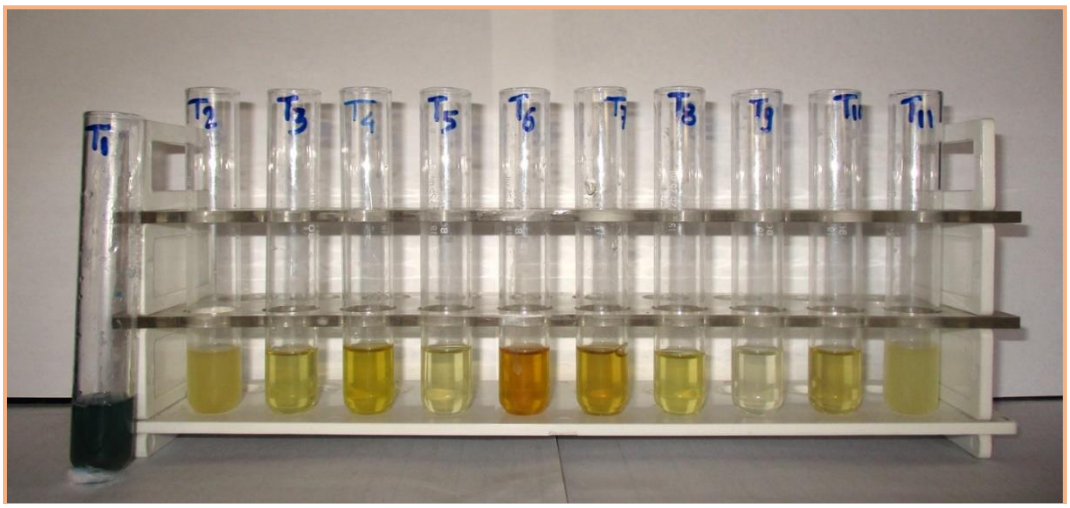


Plate 1. Siderophore production potential of selected microbial isolates on qualitative and quantitative basis

4. CONCLUSION

Iron is an essential nutrient for plant growth. Under iron deficient condition importance of siderophore is obvious and play significant role in increasing iron uptake to crop. All the tested microbial isolates are capable for producing siderophore. Among these inoculations of *Pseudomonas fluorocens* CAS medium shows maximum siderophore production followed by *Pseudomonas striata* (48.33%) and *Bacillus megaterium* (46.67%). So selection and inoculation of siderophore producing promising microbial isolates in soil or seed treatment will helpful in increasing crop production and leads to lots of saving in crop husbandry.

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SUPPLEMENTARY DATA

Supplementary data to this article can be found in this link:
https://krishikosh.egranth.ac.in/handle/1/5810187506

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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