Siderophore Production by Phosphate Solubilizing Bacteria Isolated from Heavy Metal Contaminated Soils

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Abstract

Phosphate solubilizing bacteria play a significant role in supplying phosphorus to plants and enhance the plant growth through other plant growth promotion activities such as production of siderophore, which increase the availability of iron while suppressing growth of soil-borne plant pathogens. This study was aimed at assessing the production of siderophore by phosphate solubilizing bacteria isolated from heavy metal contaminated soils. A total of 20 phosphate solubilizing bacterial strains (PSB-1 to PSB-20) were isolated from abounded mines in Boryeong area, South Korea and screened for the siderophore production using CAS agar. All isolates were found to produce siderophore in varying amounts on CAS agar plates except PSB-5, PSB-11, PSB-15 and PSB-18. The highest production was recorded after first 24 hrs of incubation implying that delayed commencement of siderophore production in all the isolates. PSB-6 and PSB-20 showed the highest siderophore production (96 % siderophore units) among the isolates,. Most of the isolates produced more than 80% of the siderophore units and they can be recommended as potential candidates for exploring their plant growth promoting characteristics and field application.

Keywords: siderophore production, phosphate solubilization, CAS agar

Introduction

Iron accounts for about 4% of the total content of minerals in the earth's crust, under aerobic conditions or in alkaline or neutral environment. Nevertheless, it occurs in the form of complexes that are refractory to solubilization, thus the element is not readily available to microorganisms and plants. Therefore, some microorganisms produce low molecular mass molecules that have high specificity for chelating iron termed siderophores. Siderophores are able to solubilize and transport ferric ion in the environment, making iron available for its frequent uptake by micro-organisms and plant. Siderophores excretion is thus considered to be one of the important features of plant growth promoting rhizobacteria (PGPR), as they could stimulate plant growth by improving iron nutrition of the plants or by inhibiting the establishment of soil-borne plant pathogens or other harmful microorganisms (McCully, 2005). The objective of this study was to assess the production of siderophores by different phosphate solubilizing bacteria isolated from heavy metal contaminated soils.

Materials and Methods

Isolation of phosphate solubilizing bacteria

Soil collected from abounded mines of Boryeong area in South Korea was used in isolating phosphate solubilizing bacteria. Serially diluted soil sample aliquots were spread on to a petri-dish with National Botanical Research Institute Phosphorus (NBRIP) medium contained 10 g glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2.6H_2O$, 0.25 g $MgSO_4.7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$ in 1 L distilled water (Nautiyal, 1999). The pH of the media was adjusted to 7. The plates were incubated for 7 days at 30 °C. The colonies with clear halos were considered to be phosphorus solubilizing colonies. Predominant colonies were further purified by re-streaking on the fresh NBRIP agar plates at 30 °C.

Screening of bacterial isolates for siderophore production

Bacterial isolates were qualitatively screened for siderophore production using chrome azurol S (CAS) blue agar according to Schwyn and Neilands (1987). The bacterial strains were inoculated on the CAS agar plates and incubated at 30 °C for 24 hrs. Orange halos around the colonies were identified as siderphore producing colonies.

Assay of siderophore production

Quantitative estimation of siderphores was done by CAS-shuttle assay (Payne, 1994). Culture supernatant (0.5 ml) was mixed with same amount of CAS reagent (0.5 ml) and absorbance was measured at 630nm against a reference consisting of equal volume of uninoculated broth and CAS reagent. Siderphore content in the aliquots were calculated using following formula.

$$\% \text{ Siderphore units} = \frac{Ar - As}{Ar} \times 100$$

Ar – Absorbance of reference

As – Absorbance of sample

Results and Discussion

The bacterial strains capable of solubilizing calcium phosphate present in NBRIP medium formed large halos in varying intensities and they were isolated as phosphate solubilizing microorganisms. A total 20 bacterial strains (PSB-1 to PSB-20) were isolated and screened for the siderophore production using CAS agar. The isolates producing siderophore showed orange halos around the colonies on CAS agar plates after 2-3 days of incubation. Except PSB-5, PSB-11, PSB-15 and PSB-18, all the other isolated strains showed the siderophore production in varying amounts on CAS agar plates (Figure 1). Interestingly,

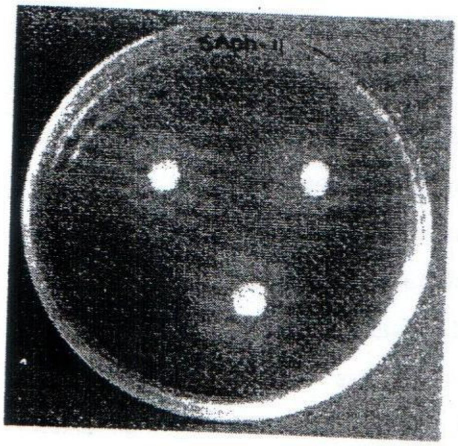

Figure 1: Orange halos produced by isolated strains on CAS agar plates due to siderophore production

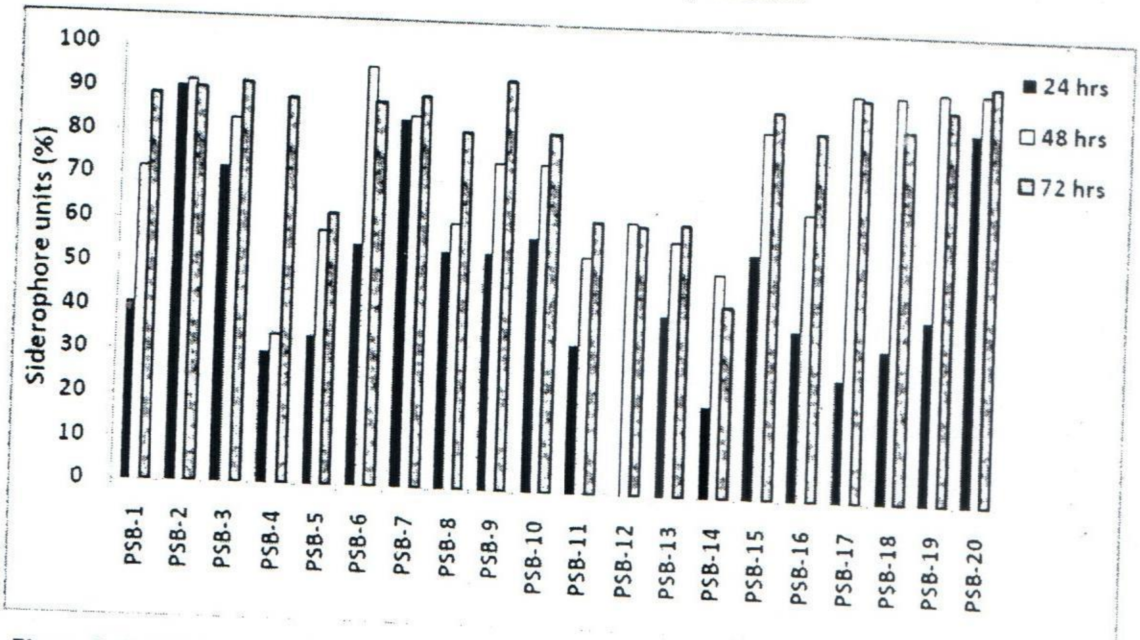

Figure 2: Siderophore production of isolated strains in liquid medium

the isolates PSB-5, PSB-11, PSB-15 and PSB-18 which couldn't produce halos on agar plates also showed siderophore production on liquid culture media (Figure 2). It has previously been reported that bacteria grown on CAS agar have failed to exhibit a halo, but have produced substantial concentrations of siderphore in a liquid medium that could be measured with CAS assay solution (Alexander and Zuberer, 1991).

Except PSB-2, PSB-7 and PSB-20, the other isolates recorded the highest siderophore production after first 24 hrs of incubation implying that they start delayed siderophore production. Most of the isolates produced more than 80 % siderophore units. Among them, isolates PSB-6 and PSB-20 showed the highest siderophore production (96 % siderophore units). The isolation of siderophore producing bacteria from metal contaminated soils has a great practical significance as they could provide nutrients to plants especially iron and reduce adverse effects of metal contaminants (Rajkumar and Freitas, 2009).Though siderophore production is recorded to be common among plant growth promoting rhizobacteria, they showed their optimum growth and siderophore production at the extreme environmental conditions such as nutrient deficient or heavy metal polluted soils. However, Hussein and Joo (2012) reported that siderophore production depends on the other soil properties as well.

Conclusion

Based on the present findings, most of the isolates can be considered as efficient siderophore producers. The efficient strains belonged to Pseudomans spp, Pantoea spp and Enterobacter spp. Research should be continued with the most efficient isolates, while increasing their population in metal contaminated soil by inoculation of soils or plants.

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