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PGPR Siderophore and Its Role in Antimicrobial Activity in Plants – A Review

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ABSTRACT

Much plant growth promoting rhizobacteria has having capability of producing siderophore. The name siderophore which is derived from iron chelating agent which prevents the entry of plant pathogens into the root system of plants and other parts of plant. Among this the symbiotic nitrogen fixing has having more efficiency of producing siderophore which induces both plant growth promotion and Inducing systemic resistance in plants. A versatile Plant growth rhizobacter (PGPR) has been described which grows on yeast extract mannitol agar. A phytohormone, siderophore has been extracted and estimated from the culture medium and appears to produce the zone of inhibition against the fungal pathogens like Aspergillus and Sclerotium.

INTRODUCTION

Iron is the fourth most abundant element in the earth's crust and the second most common metal following oxygen, silicon and aluminum respectively. Despite its relative abundance and metabolic value to most organisms, it can be a difficult nutrient to obtain. This is because when it is found in aerobic conditions and at neutral or physiologic pH, iron is oxidized to its ferric state and easily forms insoluble oxyhydroxides and other complexes that render it unavailable for metabolic use.

Microbial iron containing or iron binding compounds, most of which are classified as "Siderophores" (Greek for Iron bearers). The siderophores, as chemical entities, display considerable structural variation, the majority of them are either hydroxamates or phenolates – catecholates and all exhibited a very strong affinity for Fe (III), the formation constant lying in the range of 1030 or higher [1]. Neiland [2] reviewed the iron metabolism of microorganisms in detail. Bacterial and fungal mechanisms of iron have been discussed extensively by Lankford [3] and Emergy [4].

To overcome iron starvation, *B. japonicum* can utilize its own siderophores and those produced by other organisms [5] In assays using an iron-inefficient variety of peanuts, Jadhav et al. [6] found that the catechol siderophore of a peanut Rhizobium isolate, increased plant growth and chlorophyll content compared with plants grown with iron alone.

Siderophores are produced by PGPR under iron-limited conditions. Leeman et al. [7] reported that LPS of *P. fluorescens* strain WCS 374 and WCS 417 are the major determinants of ISR under iron-deplete conditions but under iron-limited conditions. Induction of ISR by LPS and siderophore seems to be complementary rather than additive and full induction of resistance by one determinant masks contribution by others.

PRODUCTION OF SIDEROPHORE

Siderophore production has been reported in various species of root nodulating bacteria such in fast growing *Rhizobium* spp. [8-10] and Bradyrhizobium [11].

Like other PGPR, different strains of rhizobia, i.e., *R. meliloti* [10], *S. meliloti*, *R. leguminosarum* bv. *viciae*, *R. leguminosarum* bv. *trifoli*, *leguminosarum* bv. *phaseoli*, *R. tropici* [8,12]; *Rhizobium* sp. [13] and Bradyrhizobium [5,6,13,14]; are able to produce siderophore for Fe^{3+} chelation in iron deficient environment [10,15-17].

In vitro, some strains of Rhizobium and Bradyrhizobium species have been shown, by application of variety of assays, to produce and excrete a variety of iron-cheating compounds when grown under iron-deficient conditions [15,16].

Azotobacter vinelandii produced siderophore under iron

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limiting conditions [18], the bound molybdate [19] and to be a part of iron transport systems. Reeves et al. [20] observed that increase in iron concentration increased nitrogenase activity of Azospirillum. Molybdenum starved cells reported to have reduced ex planta nitrogenase activity of *Azospirillum lipoferum* D-2 [21].

Saxena et al. [21] studied the siderophore mediated transport of molybdenum in *Azospirillum lipoferum* strain D-2. They reported that a catechol-type compound was secreted by *A. lipoferum* D-2 strain in the growth medium when the cells became molybdenum limited. The compound was identified as 3, 5-dihydroxybenzoic acid (3, 5-DHBA) which enhanced the uptake of molybdenum.

Siderophore synthesis was repressed when iron was present and induced when iron depleted from the culture medium [22]. The phenolate siderophores were mainly the amino acid conjugates of 2, 3-DHBA [23] and their presence was demonstrated in *Aerobacter aerogenes*, *Escherichia coli* and *Salmonella* sp. [23,24]. A threonine conjugate of 2, 3-dihydroxybenzoic acid in *Klebsiella oxytoga* and *E. coli* [25], a diphenolic conjugate of lysine in *Azotobacter vinelandii* [18] and threonine and glycine conjugates of 2, 3-Dihydroxybenzoic acid in cowpea Rhizobium RA-1 [26] have been reported.

CLASSES OF SIDEROPHORES

Siderophores are separated into classes based upon the chemical groups involved in iron chelation within the siderophore. The two most common classes of Siderophores are hydroxymates and phenolate-catecholates. However, several siderophores use multiple functional groups to chelate the iron and are considered mixed siderophores [27].

Hydroxymate siderophores

The hydroxymate siderophores are seen predominantly in fungi but are also produced by some bacteria. The iron chelation is provided by a hydroxymate group (-CO-N (O-)-) formed from aceylated or formylated hydroxylamines usually derived from lysine or ornithine [27]. The hydroxymate group is assembled in a two-step process, beginning with hydroxylation of the primary side-chain amine of ornithine or lysine by a flavin adenosine dinucleotide-dependent monooxygenase. The second step involves formylation by a methyl transferase, for pyoverdin and ornibactin or acetylation by an acetylase, for all other hydroxymate siderophores [27].

Phenol-catecholate siderophores

The second most common siderophore class is the phenol-catecholates, which contain a mono- or di-hydroxybenzoic acid group to chelate the iron [1]. This class of siderophores has only been observed in bacteria. The catecholate group is derived from salicylate or dihydroxybenzoic acid and the siderophores have iron binding affinities that range from very tight binding for enterobactin from *E. coli* ($Kd=10^{-52}$ M) to fairly weak binding seen in pyochelin from *P. aeruginosa* ($Kd=5 \times 10^{-5}$ M) [1].

Other classes of siderophores

Several other classes of siderophores are recognized. Citrate-hydroxymate siderophores are a mixed class of bacterial siderophores [1]. The siderophores contain derivatives of citric acid in which the distal carboxyl group has been substituted with hydroxymate groups. Another unusual class of siderophores is the mycobactins produced by *Mycobacterium* spp. of bacteria. A variety of other classes of siderophores are also known which contain various hydroxymate, catecholate and phenolate groups [1].

Crowley et al. [28] suggested that siderophores produced by root-colonizing microbes provide Fe to plants that can use the predominant siderophore types. In conjunction with transport mechanisms, ecological and chemical factors can also influence the efficacy of siderophores and phytosiderophores. They presented a model to incorporate these factors to predict conditions that may govern competition for Fe in the plant rhizosphere and observed that such competition has been a factor in the evolution of broad transport capabilities for different siderophores by microorganisms and plants. Mahmoud and Abd-Allah [29] isolated eighty four microbial isolates and tested their ability to produce siderophore and reported that among them 42 isolates exhibited positive reaction. *Pseudomonas aeruginosa* showed strongly positive reaction while Aspergillus was found to produce moderate reactions with hydroxamate assay.

Stenico et al. [30] evaluated the ability of endophytic *Methylobacterium extorquens* for siderophore production. The culture supernatants for Methylobacterium showed positive for the same and secreted hydroxamate-type of siderophores.

Simionato et al. [31] analysed the siderophore production from different strains of *Methylobacterium* spp. using capillary electrophoresis-mass spectrometry and IT mass analyzer and the analysis revealed two possible siderophore productions of Mol. wt. of 1004.3 and 798.3 Da, according to bacterial species.

Joshi et al. [32] isolated different strains of bacteria from the rhizosphere of *Arachis hypogea* (groundnut) and *Vigna radiata* (Mung bean), in which few fluorescent pseudomonads produced hydroxamates in addition to catecholates.

Lacava et al. [33] analyzed the production of siderophore production of endophytic *Methylobacterium* spp. and observed that all the strains of *Methylobacterium* spp. showed positive results for CAS assay and found to produce hydroxamate-type, but not catechol-type siderophores.

Lacava et al. [34] studied the production of siderophores by endophytic *Methylobacterium mesophilicum* and revealed

that 37 strains of the same showed positive for CAS assay, produced hydroxamate-type of siderophores.

BIO CONTROL ACTIVITY OF SIDEROPHORE

Competition for nutrients among the biocontrol bacteria and pathogen can result in the displacement of pathogen. The best understood example of the competition is the iron competition. In this, the biocontrol agent produces high Fe^{3+} affinity siderophore that sequester iron in the rhizosphere and makes it less available to certain harmful rhizospheric microorganism. The latter cannot obtain sufficient iron for growth and thus are outcompeted. Rhizobia are proficient to produce siderophores and can hamper a widely occurring plant pathogen *Macrophomina phaseolina* [10].

According to van Loon et al. [35], rhizobacterially induced salicylic acid can trigger the SAR pathway as well as ISR in some plant species. In radish, induction of systemic resistance to Fusarium wilt by two *P. fluorescens* strains WCS 374 and WCS 417 was clearly associated with the capacity of these strains to produce salicylic acid in culture [7]. The PGPR mediated ISR is often associated with the onset of defense mechanism including the increased expression of defense enzymes, such as peroxidase [36].

Stephens et al. [37] reported the ability of a bacterium to inhibit a fungal pathogen when the bacterium was grown in the laboratory on synthetic media that favored the production of either antibiotic or siderophore and determined the biocontrol activity of the bacterium *in vivo*.

The role of PPFM siderophore on disease suppression of *Fusarium* sp. and *Erwinia amylovora* was studied. Siderophore production was associated with *in vitro* inhibition of *Aphanomyces cochlioides* by strains of PPFM, but did not correlate with the ability of bacterium to suppress Aphanomyces root rot of sugar beet [38,39].

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