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The Role of Microbial Activity on Iron Uptake of Wheat Genotypes Different in Fe-Efficiency

MH. Rasouli Sadaghiani and M. Barin

Department of Soil Science, Faculty of Agriculture Urmia University, P.O.Box: 57135-165, Urmia, Iran, sadaghianii@yahoo.com

ABSTRACT

Soils in many agricultural areas have high pH, resulting in low availability of Fe. Wheat grown on such soils suffers from most micronutrient deficiencies, in particular Fe deficiency. The objective of this investigation was to determine the potentials of indigenous fluorescent Pseudomondas for siderophore production and their effects on ^{59}Fe acquisition. For this purpose, some strains of *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa* were isolated from different locations representing rhizosphere of wheat. The potentials of these strains for siderophore production were evaluated by chrome azorel-S assay (CAS blue agar) through color change. High siderophore producing Super-strains were selected for extraction of siderophores. These isolates were grown in SSM (standard succinate medium) for 72 hr at 28 $^{\circ}$C. Bacterial cell were removed by centrifugation (10000 g for 20 min) and the supernatant was filtered through filter membrane (0.22 μ) and used as crowd siderophore. Evaluation of Fe uptake and translocation were carried out with complexes of bacterial siderophores and ^{59}Fe compared with standard sierophore Desferrioxamine (DFOB) in randomized complete block design with three replications. This experiment was conducted on two wheat genotypes different in Fe-efficiency at hydroponic condition. The results showed that among the three most effective siderophores producing strains considered, the *P. putida* produced a sidrophore complex that showed efficiencies of 76 %, compared with the standard siderophore (DFOB) in the uptake of Fe and was statistically in the same group as the control. The effect of bacterial siderophores in the uptake of labeled ^{59}Fe by wheat became significant, indicating that the chemical structure of the siderophores from different strains were different. The effects of wheat genotype in ^{59}Fe activity of shoots was also significant, where the efficient Tabasi genotype contained 46 % more Fe in shoots than the inefficient Yavarous genotype. It was concluded that the siderophore complex from *P. putida* was the most effective in translocating Fe to shoots, particularly in efficient Tabasi genotype. Siderophore effectiveness in Fe availability decreased in the order;

Sid-DFOB> Sid-*putida*>Sid-*fluorescens*> Sid-*areuginosa*.

Keywords: siderophore, ^{59}Fe, wheat, Fluorescent Pseudomonads, iron-efficiency

INTRODUCTION

Soil microbial activity in rhizosphere may influence the growth of higher plants by various processes. The plant growth-promoting activity of rhizobacteria such as fluorescent pseudomonads is well-established. Several mechanisms by which plant growth-promoting rhizobacteria (PGPR) promote plant growth include the production of extracellular growth-promoting chemical substances, phytohormones, iron chelating siderophores, antibiotics and HCN which improve plants growth, reduce the population of major root pathogens, compete for energy yielding nutrients, induce plant resistance and

mineralize soil nutrients (Patten and Glick, 2002; Kloepper et al., 1980). Furthermore, PGPR have shown promise as potential biological control agents for many soil-borne root diseases (Gray and Smith, 2005).

Fluorescent pseudomanads are an important component of the rhizosphere of many plants, and are known to colonize the rhizosphere of wheat, potato, maize, grasses, pea and cucumber (Cakmakci et al., 2006; Khalid et al., 2004; Howie and Echandi, 1983; Brown and Rovira, 1976). These microorganisms improve plants efficiency in nutrients acquisition in particular iron. In calcareous soils the availability of Fe is very low due to the high pH of the soil solution and its buffering capacity that may impede Fe uptake mechanisms of many plants. Plants grown in such soils may suffer from sever Fe deficiency (Marschner et al., 1986). In order to avoid Fe deficiency, various graminaceous plants seem to rely on excretion of phytosiderophores by the roots and their uptake as a Fe complex by a highly specific uptake system that is enhanced by Fe deficiency (strategy I plants). In dicotyledonous as strategy II plants, release of protons and reducing substances combined with enzymatic splitting of chelates have been proposed as mechanisms of solubilizing soil Fe and/or uptake of chelated Fe (Marschner et al., 1986). Rhizosphere fluorescent pseudomonads are known to be antagonists to plant pathogens via siderophore production (Kloepper et al., 1980).

The efficiency of a chelate in supplying Fe to plants or microorganisms depends on its stability constants with Fe at various pH levels and competing ion concentration. Microbial siderophores form Fe complexes with high stability constants and therefore play a role in the Fe uptake by microorganisms (Neilands, 1995). Siderophores were found in soil solutions at concentrations that may influence the Fe nutrition of plants. Soil microbial activity is essential for Fe acquisition by soil-grown rape. Similarly, sorghum which is able to release phytosiderophores from the roots requires soil microbial activity to ensure satisfactory Fe supply (Rroco et al., 2003).

The aim of this study was to evaluate the effect and efficiency of pesudomonads siderophores on as Fe carrier to wheat genotypes different in Fe efficiency.

METHODS and MATERIAL

Bacterial Isolation

Soil samples were collected from 52 different locations representing rhizosphere of different wheat genotypes. Root samples were shaken vigorously to remove loosely adhering soil. The rhizosphere samples including adhering soil with root were plated on King's B-medium and the plates were incubated at 37 $^{\circ}$C for 24 h. Colonies that fluoresced under UV light (λ=356 nm) were selected and further purified on the same medium. Finally 201 strains confirmed as fluorescent pseudomonads based on biochemical tests such as arginine hydrolysis, catalase activity, production of fluorescing compounds, gelatin liquefaction and growth at 4 $^{\circ}$C and 42 $^{\circ}$C. Plant growth-promoting properties of the strains were

confirmed with their ability to produce siderophore, indole acetic acid and phosphate solubilization. The potentials of these strains for siderophore production were evaluated by chrome azorel-S assay (CAS blue agar) through color change (Schwyn and Neilands, 1987). Among the isolates, 3 super-strains (high siderophore-producing strain) belong to different species of *Pseudomonas* sp (*P. putida* FP159, *P. fluorscens* FP73 and *P. aeruginosa* FP35) were selected for subsequent experiment.

Collection of Siderophores

The isolates were grown in standard succinate medium (SSM) consisting of (g l^{-1} distilled water): K_2HPO_4, 6.0; KH_2PO_4, 3.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4$ $7H_2O$, 0.2; succinic acid, 4.0. The pH adjusted to 7 by addition of NaOH before sterilization (Meyer and Abdallah, 1978). Cultures were grown at 28 °C for 72 h with shaking. After 72 h, the cultures medium had turned yellow-green indicating production of siderophores (Meyer and Abdallah, 1978). Bacterial cells were removed by centrifugation at 10000g for 20 min. The supernatants were filter sterilized through 0.22 µm membrane filter. The filtrates were kept in frizzier and used as crude siderophore source.

Preparation of ^{59}Fe-Siderophores (FeSid) Complexes

Standard sierophore (DFOB) was obtained from Sigma. The bacterial siderophores prepared as described above. ^{59}FeFOB and ^{59}FeSid were prepared by dissolving the ligands in distilled water and adding appropriate molar amounts of $^{59}FeCl_3$ in dilute HCl. Solution were stirred until dissolved and the pH was adjusted to ca. 5.8 with 10 mM 2-N- morpholino ethanesulfonic acid (MES). FeSids were used for experiments immediately after preparation (Johnson et al., 2002).

^{59}Fe Uptake and Transport

Evaluation of Fe uptake and translocation were carried out with complexes of bacterial siderophores and ^{59}Fe compared with DFOB in randomized complete block design with three replications. These processes was done by placing the intact root system of 8 wheat seedlings in 100 ml of nutrient solution, additionally containing 10 mM MES at pH of 5.8 and 10 µM ^{59}Fe labeled chelates (1.11 Bq mol^{-1}Fe). Nutrition solution containing: 2 mM $Ca(NO_3)_2$, 0.25 mM KH_2PO_4, 0.1 mM KCl, 0.88 mM K_2SO_4, 1 mM $MgSO_4$ 7H2O, 1 µM H_3BO_3, 0.2 µM $CuSO_4$ $5H_2O$ and 0.2 µM $(NH_4)_6MoO_{24}$. Wheat genotypes had different Fe efficiency, Tabasi was Fe-efficient and Yavarous was Fe-inefficient in terms of phytosiderophore producing (Rasouli Sadaghiani et al., 2007). Seedlings were maintained under growth chamber condition with aeration in glass pots for 6 h. At the end of experiments, roots were washed with distilled water, followed by three washes in nutrient solution without Fe and containing 40 µM Na_2EDTA, followed by a final distilled water rinse. Each plant was separated into roots and shoots. After air drying, ^{59}Fe was measured using a gamma counter (Johnson et al., 2002).

RESULTS

Bacteria in the Rhizosphere of Wheat

Evaluation of wheat rhizosphere from 10 provinces of Iran showed to included 201 fluorescent pseudomonads isolates; among them, 53%, 44% and 3% were *P. putida*, *P. fluorescens* and *P. aeruginosa*. These results have some similarities with those found elsewhere for rhizosphere of lemon (Gardner et al., 1984) and maize (Lalande et al., 1989).

Plant Growth-Promoting Properties

Up to 92% of isolated strains produced IAA and detected by the Salkowski reagent under colorimetry, in the range 2.13 mg l^{-1} to 26.98 mg l^{-1}. The highest concentration of IAA was obtained from *P. putida* FP159. Most of *putida* species had higher ability to produce IAA compared to other species.
All 201 strains formed colony on CAS blue agar and produced siderophore at different level. Siderophore production by the *Pseudomonas* species, isolated from King B liquid medium can be seen in Fig. 1.

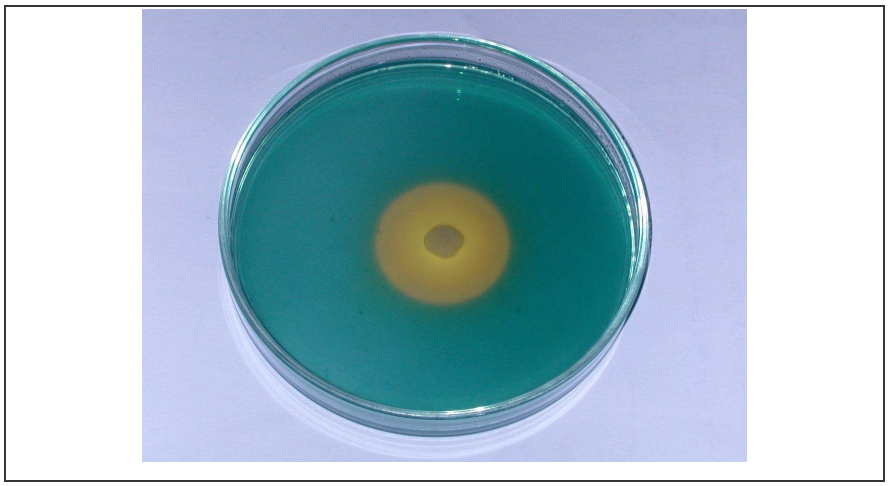

Fig. 1. Siderophore production by *Pseudomonas putida*

The results showed that among the three most effective siderophores producing strains considered, the *P. putida* produced a sidrophore complex that showed efficiencies of 76% in the uptake of Fe, compared with the standard siderophore (DFOB) and was statistically in the same group as the control (Table 1).

Table 1. Activity of ^{59}Fe in shoots and roots of wheat

Siderophore complex	^{59}Fe Activity (Bq/gDW)	
	Shoot	Root
^{59}FeFOB	0.446	0.441
^{59}Fe Sid- *putida*	0.341	0.739
^{59}FeSid- *fluorescens*	0.311	0.243
^{59}Fe Sid- *aeruginosa*	0.130	0.261
$LSD_{0.05}$	0.124	0.293

^{59}Fe activity of shoot in FOB complex was higher than pseudomonads siderophore complexes. However, this difference was not significant in case of siderophore complex of *putida*. Desferrioxamine B (DFOB), a siderophore produced by *Streptomyces pilosus* was shown to form stable Fe complex (FOB) in nutrient and soil solutions between pH 4 and 10 (Cline et al., 1982). The effect of bacterial siderophores in the uptake of labeled ^{59}Fe by wheat became significant, indicating that the chemical structure of the siderophores from different strains may be different. The effects of wheat genotype in ^{59}Fe activity of shoots was also significant, where the efficient Tabasi genotype contained 46% more Fe in shoots than the inefficient Yavarous genotype. Rasouli Sadaghiani et al. (2007) showed that Tabasi as bread wheat had high efficiency in terms of phytosiderophores release at Fe deficiency condition. In contrast, Yavarous as durum wheat was sensitive to Fe deficiency and produced very low amount of phytosiderophores at same condition.

Commercial Fe chelates as well as phytosiderophores have shown high efficiency in Fe uptake compared to siderophores. Reid et al. (1984) showed that the siderophore ferrichrome was a more efficient Fe carrier than FeEDDHA for oat. However there are controversy findings. Fe uptake from ^{59}Fe-siderophore was lower than uptake from ^{59}FeEDTA by cucumber and ^{59}Fe-phytosiderophore by maize. Siderophore complex of Fe could be effective in Fe uptake indirectly, by increasing the extracellular supply of Fe at the root surface (Walter et al., 1994). It was concluded that the siderophore complex from *P. putida* was the most effective in translocating Fe to shoots, particularly in efficient Tabasi genotype. Siderophore effectiveness in Fe availability decreased in the order;
Sid-DFOB> Sid-*putida*>Sid-*fluorescens*> Sid-*areuginosa*.

DISCUSSION

In higher plants two distinct strategies for Fe acquisition exist under condition of limited Fe supply (Marschner et al., 1986). The operation of strategy I depends on the supply of soluble Fe to the Fe reductase system at the plasma membrane of the rhizodermal cells (Romheld et al., 1987). Siderophores can increase the concentration of Fe at the uptake sites of the roots by increasing the solubility and mobility of Fe in the soil. Plant with strategy II, produce phytosiderophores. In this study, all strains formed colony on CAS blue agar and produced siderophore at different level (Fig. 1). In the Fe nutrition of strategy II plants, the role of microbial siderophores depends on their stability and thus on their ability to supply readily-accessible inorganic Fe^{3+} to the extracellular Fe pool (root surface and apoplasm) for the chelation by phytosiderophores released by the roots (Romheld and Marschner, 1986). In this study siderophore complex of *P. putida* showed high efficiency in Fe uptake compared to other species. Crowley et al. (1988) introduced a microbial siderophore-Fe transport system in oat. Our results suggest operation of heterologous ionophore uptake in Tabasi as efficient genotype. Similar results were observed

in studies of Sharma et al. (2003). More recently, Fernandez et al. (2005) obtained evidence of plant Fe utilization after foliar treatment with microbial siderophores.

Sorghum and sunflower grown under Fe deficient condition in nutrient solutions were able to utilize Fe from the FOB complex (Cline et al., 1982). Reid et al. (1984) showed that the siderophore ferrichrome was a more efficient Fe carrier than FeEDDHA for oat grown in nutrient solution. In soil system a preliminary study showed that ferrated pseudomonad siderophores are active in the remedy of lime-induced chlorosis (Jurkevitch et al., 1988). Fe complex of *putida* let to increase Fe concentration of roots in contrast to the effects of other complexes as well as FeFOB (Table 1). Theses accumulated Fe serve as Fe apoplasmic pool and may involve in ligand exchange phenomena. In this hypothesis which is presented by Yehuda et al. (1996), Fe from Fe-siderophores is taken up by strategy II plants via an indirect mechanism that involves ligand exchange between the ferrated microbial siderophore and phytosiderophores, which are taken by the plants. Tabasi genotype has shown to produce large amount of phytosiderophores on Fe deficient condition (Rasouli Sadaghiani et al. 2007).

Several researches show that microbial inoculation enhances plant Fe uptake. Masalha et al. (2000) stated that plants (maize and sunflower) cultivated under non-sterile condition grew well, showed no Fe deficiency symptoms and had fairly high Fe concentration in the roots in contrast to plant grown in the sterile medium. It may be therefore assumed that microbial activity in particular their siderophores is of pivotal importance for plant Fe uptake.

The data presented in this study explores microbe-plant interaction in terms of iron uptake from particularly the insoluble oxide form of iron and supports the mechanisms of heterologous iron uptake in wheat system via microbial siderophores. For calcareous soils which prevalent in Iran, efficient strains of pseudomanads like studied strains here will be of great interest to combat iron chlorosis and additionally improve strategic crop yield.

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