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Differential Siderophore Utilization and Iron Uptake by Soil and Rhizosphere Bacteria

EDOUARD JURKEVITCH,[1] YITZHAK HADAR,[1]* AND YONA CHEN[2]

Department of Microbiology and Plant Pathology[1] and Department of Soil and Water Sciences,[2] Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

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The differential availabilities of the hydroxamate siderophores ferrioxamine B (FOB) and ferrichrome (FC) and the pseudobactin siderophores St3, 7NSK$_2$, and WCS 358 as sources of Fe for soil and rhizosphere bacteria were studied. About 20% of the total bacterial CFU from the rhizospheres of four plant species were able to use FOB as the sole Fe source in an Fe-deficient medium, while about 12, 10, 2, and >1% were able to use FC and pseudobactins 7NSK$_2$, St3, and WCS 358, respectively. Of the 165 colonies isolated from plates containing pseudobactins, 64 were able to use the pseudobactin on which they were isolated as the sole Fe source in pure culture. Cross-feeding tests showed that almost all of these 64 strains were also able to use at least one of the other siderophores studied (pseudobactin, FOB, or FC). *Pseudomonas putida* StS2, *Pseudomonas maltophilia* 7NM1, and *Vibrio fluvialis* WS1, which were originally isolated on pseudobactins St3, 7NSK$_2$, and WCS 358, respectively, were selected for their ability to grow with pseudobactin St3 as the sole Fe source. They incorporated $^{55}Fe^{3+}$ mediated by pseudobactin St3 at various rates (71.5, 4, and 23 pmol/min/mg [dry weight] of cells, respectively). Similarly, *P. putida* St3 was shown to incorporate $^{55}Fe^{3+}$ mediated by FOB and FC. We suggest that the ability of bacteria to utilize a large variety of siderophores confers an ecological advantage.

Fe forms insoluble hydroxides at neutral and basic pHs (20). Microorganisms have therefore evolved efficient uptake mechanisms to obtain sufficient amounts of this essential element (26). Most aerobic and facultative aerobic bacteria possess a high-affinity Fe transport system in which siderophores are excreted and the consequent Fe complex is taken up via the cognate-specific receptor and a transport pathway (27). Many microorganisms are also able to utilize the Fe^{3+} complexes of siderophores which they have not synthesized (8). The importance of Fe is reflected by the number of mechanisms developed by microorganisms for its acquisition, which include a membrane-bound chelator (31), reduction of Fe chelates (10, 19), and an unknown mechanism in *Serratia marcescens* (38) in addition to those already mentioned.

Fluorescent pseudomonads produce pseudobactin siderophores. The main differences observed between those produced by different strains are the number, composition, and sequence of their L and D amino acids, which are thought to give the molecules their receptor specificities (14). Siderophores produced by several of the fluorescent *Pseudomonas* spp. play a role in the biological control of plant pathogens and in plant growth promotion through competition for Fe (16, 33). The excreted pseudobactin chelates Fe because it has a higher affinity for Fe than do the siderophores from most microorganisms that are deleterious to plant growth (32). However, this explanation does not take into account the proton dissociation constants and respective concentrations of the siderophore species involved, nor does it consider the kinetics of exchange, all of which are thought to be limiting factors (5).

A question pertinent to the ecology of the rhizosphere with respect to Fe competition between various microorganisms is the availability of the excreted siderophores to other species. Microorganisms exhibit tremendous diversity in their ability to utilize exogenous siderophores. Receptors for the iron-ferrichrome (FeFC) complex have been detected in *Escherichia coli* and in *Erwinia chrysanthemi* (3, 11). In *E. chrysanthemi* and in *Pseudomonas aeruginosa*, the receptor for enterobactin is also present (11, 28). Certain isolates of *Rhizobium trifolium* grow by using various siderophores (35), and *Rhodopseudomonas sphaeroides* is able to incorporate parabactin (24). *Pseudomonas putida* WCS 358 harbors a receptor responsible for the uptake of pseudobactin WCS 358 and at least two other outer membrane proteins, enabling it to use many other pseudobactin siderophores (18). It has been shown that in peanut plants grown in an Fe-deficient soil which has been amended with Fe^{3+}-pseudobactin, the proportion of fluorescent pseudomonads in the rhizosphere increases (15). To date, auxotrophic bacteria have been used in most studies aimed at detecting hydroxamate siderophores in various environments (1, 2, 29). The aim of this study was to evaluate the availability of various siderophores as Fe sources for soil and rhizosphere microfloras. Emphasis was placed on the hydroxamate siderophores ferrioxamine B (FOB) which is produced by *Streptomyces pilosus*, and FC, which is produced by many fungi, and on the pseudobactin siderophores of three different fluorescent pseudomonads.

MATERIALS AND METHODS

Bacterial strains and growth media. *P. putida* St3 was isolated from the peanut rhizosphere (15). *P. putida* WCS 358 (12) and *P. aeruginosa* 7NSK$_2$ (14a) were kindly provided by P. Weisbeek (Utrecht, The Netherlands) and by M. Höfte (Ghent, Belgium), respectively. The bacteria were grown in liquid modified King's B (LMKB) medium (13). For the rhizosphere and soil microbial population experiments, appropriate dilutions were plated on (i) MKB medium; (ii) MKB medium amended with 1 mg of EDDHA (ethylenediaminedi-*o*-hydroxyphenylacetic acid, Sigma, St. Louis, Mo.), deferrated as described by Rogers (30), per ml (this

* Corresponding author.

medium contains no available Fe and served as a negative control); and (iii) MKB medium plus EDDHA (MKB+EDDHA medium) (1 mg/ml) amended with the Fe^{3+} complex of FC, FOB, pseudobactin St3, pseudobactin WCS 358, or pseudobactin $7NSK_2$ to a concentration of 5×10^{-6} M (this medium was used for the quantification of the siderophore-utilizing microorganisms). Rhizosphere medium with and without EDDHA was prepared as described by Buyer et al. (6).

Purification of the siderophores. Pseudobactin St3 was purified from *P. putida* St3 as previously described (15). Pseudobactin from strain $7NSK_2$ was purified in essentially the same way. Ion-exchange chromatography on CMC-25 yielded seven bands and a front-running fraction. The first two fractions collected were brown and able to sustain the growth of $7NSK_2$ cells plated on MKB+EDDHA medium. Pseudobactin from *P. putida* WCS 358 was purified as described by van der Hofstad et al. (36).

The pseudobactin siderophores were further purified by a high-performance liquid chromatography (HPLC) method based on the method developed by Buyer and Leong (4) with modifications, by using an LKB 51 system (LKB Products, Bromma, Sweden) with a 5-μm LiChroCART 250-4 RP-18 column (4 by 250 mm) (Merck, Darmstadt, Germany). Peaks were detected with a Uvitec UV detector (Jasco, Tokyo, Japan) at 280 nm and a V15 detector (Pharmacia, Uppsala, Sweden) at 400 nm. Purity was determined from the area under the peaks. When a linear water-acetonitrile gradient (1 to 69% acetonitrile) was used at a flow rate of 0.98 ml/min, pseudobactin St3 yielded one major peak which accounted for 75% of the total area under the peaks. This peak was collected for use in the experiments. Pseudobactin $7NSK_2$ was analyzed with a linear water-acetonitrile gradient (5 to 45% acetonitrile) at a flow rate of 0.7 ml/min. The purity of the injected material was greater than 90%. FC and ferrichrome A (FCA) were purified according to the method of Crowley et al. (7). Triacetylfusarinine, ferrirubin, ferricrocin, and ferrichrysin were kind gifts from H. Anke (Universität Kaiserslautern, Kaiserslautern, Germany). FOB (Desferal) was purchased from Ciba-Geigy (Basel, Switzerland).

Siderophore utilization by rhizosphere microflora. Two dicots (bean [*Phaseolus vulgaris*] and melon [*Cucumbis melo*]) and two monocots (sorghum [*Sorghum bicolor*] and maize [*Zea mays*]) were grown in conical test tubes 2.8 cm in diameter and 11.5 cm long. The tubes were filled with 40 g (air dry weight) of a Mitzpeh Massua mountain renzina soil (loamy clay; Xerorthent) containing 630 g of $CaCO_3$ per kg and 1.5 mg of DTPA (diethylenetriamine pentaacetic acid)-extractable Fe per kg. The test tubes were sliced in half lengthwise to enable gentle separation of the roots from the soil at the end of the growth period. The plants were grown in a growth chamber at 28°C under an 18-h light/6-h dark photoperiod. After 10 to 14 days, excess soil was washed from the roots in a sterile physiological solution (0.8% NaCl). The roots were then gently shaken in 50 ml of physiological solution in Erlenmeyer flasks containing glass beads in order to leave only the tightly adhering soil particles and the clots formed with the fine roots. The roots were drained of excess water on sterile filter paper, cut, mixed, and weighed. Samples were taken for dry weight determinations. The remainder of the root mixture was homogenized with a mortar and pestle, and 10-fold serial dilutions in physiological solution were made and plated on the media described above. The MKB plates were counted after an incubation period of 18 to 24 h at 30°C; the MKB+EDDHA plates with or without added Fe-siderophores (as described above) were counted after 48 to 60 h at the same temperature.

Siderophore utilization by soil microfloras. Various soils (10 g [air dry weight] each of mountain renzina from an open field, mountain renzina from under a forest canopy, and red-brown Rehovot sandy loam from an open field) were shaken in 90 ml of physiological solution for 4 h. Tenfold serial dilutions were plated on the media described above and incubated for 48 to 72 h at 30°C.

Cross-feeding experiments. Ninety-eight, 51 and, 20 colonies of bacteria grown on plates containing pseudobactins St3, $7NSK_2$, and WCS 358, respectively, were isolated and stored in 15% glycerol at −70°C (23). The bacteria were grown overnight in LMKB medium, and appropriate dilutions were plated onto MKB+EDDHA agar. Ten microliters of a 10^{-4} M solution of Fe^{3+}-siderophore complexes (pseudobactin St3, $7NSK_2$, or WCS 358) was dropped onto 6-mm Whatman paper disks placed on the agar. Pseudobactins used for this experiment were purified by HPLC. Isolates capable of growing as pure cultures on MKB+EDDHA medium amended with the pseudobactin used for their isolation were tested for their ability to use the two other pseudobactins, FOB and FC. Bacteria were grown and plated as described above, 1 ml of the medium was centrifuged, and the supernatant was tested for the presence of siderophores by the chrome azurol S (CAS) universal assay (34). The plates were incubated for 48 h at 28°C. In control treatments, distilled water and a 10^{-4} M solution of FeEDDHA were used instead of the siderophores.

^{55}Fe uptake. The bacteria were grown in LMKB medium to mid-log phase (A_{600}, 0.6 to 0.7), centrifuged for 15 min at 2,500 rpm in a Centrikon H-401 centrifuge with an A8-2h rotor (Kontron-Hermle, Zürich, Switzerland) at room temperature, and resuspended in half-strength growth medium to a final A_{600} of 0.3. This suspension was incubated for 30 min in a water bath before the addition of the $^{55}Fe^{3+}$-labelled complex.

At the beginning of the experiment, a volume of the labelled stock solution was added to the incorporation medium, bringing the Fe^{3+}-siderophore complex to a final concentration of 10^{-6} M. Aliquots (0.6 ml) were taken in duplicate at different time intervals, layered onto a silicon oil mixture, and centrifuged as described by de Weger et al. (9). All data were corrected for cell growth during the assay period. Counting was performed with LS1801 counter (Beckman Instruments, Inc., Fullerton, Calif.).

Statistical analysis. Three independent plant experiments were performed in triplicate for each plant species. Since the error variances of the separate analyses of variance were homogeneous, data were pooled and analyzed as the means of the nine resulting determinations. Data were logarithmically transformed and statistically analyzed by analysis of variance; means were separated by Duncan's multiple-range test ($P = 0.05$).

RESULTS

The proportion of rhizosphere bacteria able to produce colonies with different microbial Fe chelates as the sole Fe source was estimated. No growth was observed in the MKB+EDDHA control medium. This medium was amended with different types of Fe-siderophores and compared with the MKB medium. The two Fe-hydroxamate siderophores tested (FC and FOB) and pseudobactin $7NSK_2$ sustained growth of a significantly higher proportion of rhizosphere CFU than the pseudobactin siderophores St3

TABLE 1. Number of rhizosphere bacterial CFU obtained on MKB+EDDHA plates amended with various siderophores[a]

Treatment	No. of CFU obtained from the following plant[b]:			
	Maize	Sorghum	Melon	Bean
Control	8.5×10^7A	5.25×10^7A	1.44×10^8A	2.95×10^8A
FOB	1.74×10^7B	1.4×10^7B	2.2×10^7B	7.1×10^7B
FC	1.2×10^7B	6×10^6BC	1.48×10^7BC	4.7×10^7B
$7NSK_2$	9.3×10^6B	3.8×10^6BC	8.7×10^6BC	4.35×10^7B
St3	1.35×10^6C	1.15×10^6C	4.3×10^6C	9.8×10^6C
WCS 358	1.95×10^6C	1.6×10^5D	1.25×10^6D	1.55×10^6D

[a] The Fe-siderophore complex was added to the plating medium at a final concentration of 5×10^{-6} M.
[b] Within each column, values followed by the same letter are not significantly different for $P = 0.05$.

and WCS 358 (Table 1). In sorghum, maize, melon, and bean rhizosphere cultures, 27, 20.5, 15, and 24%, respectively, of the total bacterial counts from the MKB medium could develop colonies when FeFOB was added to the MKB+EDDHA medium. The proportion of CFU that could use FeFC was lower (11.4, 14.2, 10.1, and 15.8%, respectively). The Fe^{3+}-pseudobactins were much less available, but the proportion of bacteria able to use those siderophores varied for the three pseudobactins studied. Fe^{3+}-pseudobactin $7NSK_2$ was used as an Fe source by a greater proportion of the total CFU (6 to 14%) than the siderophores produced by strains St3 (1.5 to 3%) and WCS 358 (0.3 to 2.5%).

Three different bulk soils were tested. In open-field mountain renzina soil, 27, 6.3, and 0.5% of the total CFU grew on the FeFOB-, FeFC-, and Fe-pseudobactin St3-amended plates, respectively. The same trend was apparent (Table 2) when the forest canopy-covered mountain renzina soil and Rehovot sandy loam were studied.

These results could be misleading, as it was observed that growing colonies could promote the development of more colonies, probably through the excretion of metabolites. Therefore, a total of 165 isolates were randomly reisolated from plates containing Fe-pseudobactins (St3, $7NSK_2$, and WCS 358). These isolates were used to determine the extent to which their growth reflected the use of the siderophore tested and not interactions between colonies. These isolates were tested as pure cultures for their ability to use the Fe-siderophore on which they were originally isolated. To avoid misinterpretation, the pseudobactin siderophores were purified by HPLC. Of the 96, 49, and 20 isolates from plates containing the Fe-siderophores of strains St3, $7NSK_2$, and WCS 358, respectively, 45, 15, and 4 (a total of 64) grew as pure cultures on plates containing the same pseudobactin.

TABLE 2. Number of soil bacterial CFU obtained on MKB+EDDHA plates amended with various siderophores[a]

Treatment	No. of CFU obtained from the following soil[b]:		
	Red-brown sandy loam	Canopy-covered mountain renzina	Open-field mountain renzina
Control	2.9×10^6A	3.8×10^7A	8.4×10^6A
St3	2.1×10^3C	2.1×10^5C	4×10^4D
FOB	5.8×10^5B	4.25×10^6B	2.2×10^6B
FC			5.3×10^5C

[a] The Fe-siderophore complex was added to the plating medium at a final concentration of 5×10^{-6} M.
[b] Within each column, values followed by the same letter are not significantly different for $P = 0.05$.

TABLE 3. Pattern of siderophore utilization by strains isolated on pseudobactins St3, $7NSK_2$, and WCS 358

Strain or group	No. of strains	Utilization of siderophore[a]:				
		St3	$7NSK_2$	WCS 358	FOB	FC
1	22	+	+	+	+	+
2	21	+	+	−	+	+
3	5	+	−	−	+	−
4	3	+	−	−	+	+
5	2	+	+	−	+	−
6	2	+	+	−	−	+
7	2	+	−	−	−	+
8	2	−	−	+	+	−
9	2	−	+	+	+	+
10	1	+	+	+	−	−
11	1	+	−	−	−	−
12	1	+	+	−	−	−
P. putida St3		+	+	−	+	+
P. aeruginosa $7NSK_2$		−	+	−	+	+
P. putida WCS 358		+	+	+	+	+

[a] Bacteria were tested for growth around paper disks that were impregnated with 10 μl of a 10^{-4} M solution of the Fe-siderophore complex and placed on top of MKB+EDDHA plates. +, growth; −, no growth.

These 64 strains were then tested in cross-feeding experiments for their ability to utilize the five Fe-siderophores (Table 3). The strains could be divided into three main groups and nine smaller ones on the basis of their patterns of siderophore utilization. Groups 1 and 2, which comprised two-thirds of the strains, differed only in their utilization of Fe-pseudobactin WCS 358 (Table 3). Group 3 comprised five strains that were able to grow only on Fe-pseudobactin St3 and FeFOB. Growth of the remaining 16 isolates followed nine different patterns (groups 4 to 12). Fe-pseudobactins St3, $7NSK_2$ and WCS 358 could be used by 60, 51, and 27 of the tested strains, respectively. Thus, 15 strains that were isolated on another pseudobactin could use pseudobactin St3. Similarly, 36 and 23 strains that were not isolated on pseudobactins $7NSK_2$ and WCS 358, respectively, could use these siderophores. Of the 64 strains isolated for their ability to use a certain Fe-pseudobactin, 57 and 52 were also able to utilize the Fe-hydroxamate siderophores FeFOB and FeFC, respectively. In a few cases, poor and delayed growth was observed on the water and FeEDDHA control plates, as opposed to larger and faster-growing colonies of the same strains in the presence of a utilizable siderophore. This rules out the possibility that ligand exchange occurs, as the only siderophore available for growth stimulation is the tested compound. The ability of those isolates to produce siderophores was tested by the CAS assay (34). Seventy-five percent of the isolates had a positive reaction to the CAS test, although of the 16 isolates originating from the soil experiments, 11 were either CAS negative or reacted only very slightly. The pattern of siderophore utilization by pseudomonad strains St3, $7NSK_2$, and WCS 358 showed that all three strains were able to utilize FeFOB and FeFC (Table 3). *P. putida* WCS 358 was also able to use the siderophores of the two other pseudomonads. *P. putida* St3 could use pseudobactin $7NSK_2$ but not pseudobactin WCS 358, and *P. aeruginosa* $7NSK_2$ could grow only on its own siderophore (Table 3). Utilization of a variety of Fe-hydroxamate siderophores by these three strains was assessed with rhizosphere medium amended with EDDHA (Table 4).

TABLE 4. Utilization of Fe-hydroxamate siderophores by three *Pseudomonas* strains in rhizosphere medium plates amended with EDDHA

Siderophore	Radius of growth (mm) for strain[a]:		
	P. putida		*P. aeruginosa* $7NSK_2$
	St3	WCS 358	
Triacetylfusarinine	0	10	0
Coprogen B	6	10	11
FOB	11	12	15
FC	8	10	14
FCA	9	0	0
Ferrirubin	9	0	12
Ferricrocin	8	11	14
Ferrichrysin	12	12	12

[a] Radius of growth from the edge of disks impregnated with 10 μl of a 10^{-4} M solution of the tested Fe-siderophore complex after an incubation period of 18 h at 28°C.

FeFC, Fe-ferricrocin, Fe-ferrichrysin, FeFOB, and Fe-coprogen B supported strong growth of all three strains. Only strains WCS 358 and St3 utilized Fe-triacetylfusarinine and FeFCA, respectively, while strains $7NSK_2$ and St3 but not WCS 358 utilized Fe-ferrirubin.

The ability of a certain siderophore to supply Fe to a microbial cell in the rhizosphere is certainly determined by the presence and affinity of an uptake system for the siderophore's Fe complex, among other parameters. *P. putida* StS2 (which was isolated on Fe-pseudobactin St3-containing plates) and *Pseudomonas maltophilia* 7NM1 and *Vibrio fluvialis* WS1 (which were isolated from plates containing the Fe-pseudobactins $7NSK_2$ and WCS 358, respectively) were all able to utilize Fe-pseudobactin St3 and to incorporate ^{55}Fe-pseudobactin (Fig. 1). Uptake rates in the linear section of the curve were calculated and were found to vary widely among the strains. The pseudobactin St3-mediated $^{55}Fe^{3+}$ uptake rates were 89, 72, 23, and 4 pmol/min/mg (dry weight) of cells in strains St3, StS2, WS1, and 7NM1, respectively (Fig. 1 and 2). The final Fe content varied greatly, with strain St3 incorporating 1.2, 3, and 24 times more Fe after 20 min than isolates StS2, WS1, and 7NM1, respectively.

Uptake of $^{55}Fe^{3+}$ in *P. putida* St3 mediated by the

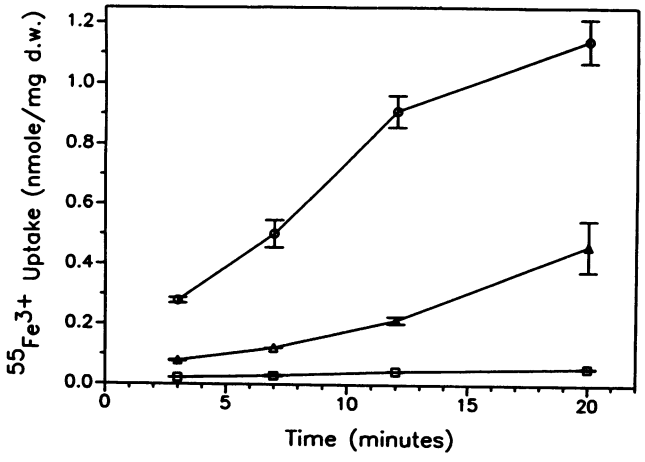

FIG. 1. Uptake of $^{55}Fe^{3+}$ (10^{-6} M) mediated by pseudobactin St3 by strains StS2 (○), 7NM1 (□), and WS1 (△). d.w., dry weight. Error bars represent standard error.

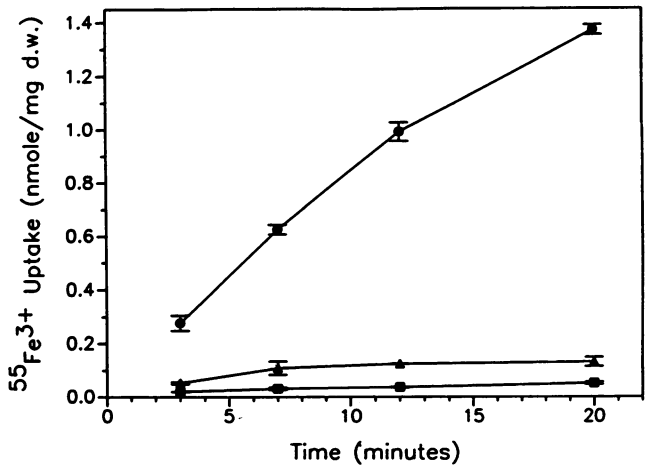

FIG. 2. Uptake of $^{55}Fe^{3+}$ (10^{-6} M) mediated by pseudobactin St3 (●), FOB (▲), and FC (■) by *P. putida* St3. Error bars represent standard error.

hydroxamate siderophores FOB and FC was measured (Fig. 2). These hydroxamates mediated the uptake of $^{55}Fe^{3+}$ at relatively low levels compared with that of pseudobactin St3-mediated uptake, with rates of 15.5 and 4.4 pmol/min/mg (dry weight) of cells for FOB and FC, respectively. The final Fe concentrations in the cells were 1.37, 0.132, and 0.052 nmol/mg (dry weight) of cells for pseudobactin St3-, FOB-, and FC-mediated $^{55}Fe^{3+}$ uptake, respectively.

DISCUSSION

It has often been assumed that competition for Fe in the rhizosphere is controlled by the Fe affinity of the siderophores, whereby the ligands produced by the biocontrol agent have higher formation constants than those of the pathogen (22, 32). Other important factors include the concentrations of the various siderophores involved, the kinetics of exchange, and the availability of the Fe complexes to microorganisms that are present (5). Very little information about these other factors, which may be decisive in the outcome of the competition, is available.

In this report, we show that a significant proportion of rhizosphere and soil bacteria are able to utilize the Fe complexes of the hydroxamate siderophores FOB and FC as an Fe source. Two of the pseudobactin-type siderophores that were tested could sustain the growth of a low proportion of the microbial population. Although the number of CFU varied, the patterns of Fe-siderophore availability were similar for the four plant species studied, as well as for the three soils studied. These data emphasize the importance of the differential availability of the siderophores produced by various microorganisms in the rhizosphere.

About 40% of the colonies isolated on plates containing a certain type of pseudobactin were subsequently able to use the same pseudobactin as an Fe source. It is assumed that the remaining colonies used metabolites excreted by other colonies to initiate growth (21). Many isolates that could use Fe-pseudobactin St3 could also use Fe-pseudobactin $7NSK_2$, and vice versa. The Fe-hydroxamate siderophores FOB and FC could be utilized by most of the 64 strains isolated on a pseudobactin siderophore. Cross-feeding might be a widespread phenomenon in soil and rhizosphere bacteria, with the ability to scavenge the siderophores produced

by other microorganisms conferring an ecological advantage. In a study involving fluorescent *Pseudomonas* spp., Buyer and Leong (4) showed that beneficial and deleterious strains that could utilize a specific exogenous Fe-pseudobactin were not inhibited by the producing strain. In the present study, it was shown that three strains of fluorescent pseudomonads utilized Fe-hydroxamate siderophores that have very different structural features and that are produced by a wide range of fungi (17). The mechanisms involved in Fe uptake mediated by these siderophores are unknown, but recognition and uptake probably occur via receptors.

It has been previously shown that about 10% of the bacterial population in the rhizosphere of maize grown in a Belgian soil that was not deficient in Fe was able to use FeFOB as the sole Fe source (13). However, only about 0.3% of the general population could use the Fe-pseudobactin $7NSK_2$, a much lower percentage than that found in the present study. This difference may be due to the fact that, in the former study, the supplemented pseudobactin $7NSK_2$ was only partially purified. Since *P. aeruginosa* $7NSK_2$ also produces the siderophore pyochelin and the phenazine pyocyanin (13), the extract might have contained those compounds, which, in turn, may have hindered the growth of various microorganisms. On the other hand, the observation that more bacteria from the rhizosphere of plants grown in Mitzpeh Massua mountain soil than from the rhizosphere of plants grown in soils not deficient in Fe were able to utilize Fe mediated by various siderophores raises the pertinent possibility that microbial populations from Fe-deficient environments have more diverse or more efficient mechanisms of obtaining Fe than microorganisms that dwell in an Fe-rich environment.

Three strains that were able to utilize Fe-pseudobactin St3 in the MKB+EDDHA plates showed very different rates of pseudobactin St3-mediated $^{55}Fe^{3+}$ uptake. Similarly, pseudobactin St3 mediated uptake of $^{55}Fe^{3+}$ by *P. putida* St3 much more efficiently than FOB and FC. Uptake rates of $^{55}Fe^{3+}$ mediated by FOB and FC were in a range similar to that reported for *S. pilosus* and *E. coli*, respectively (25, 37).

Competition for Fe can be regarded as occurring in two stages: (i) competition between the excreted siderophores for the metal and (ii) competition between microorganisms for the Fe-siderophore complexes. The former is controlled by proton dissociation and formation constants of each siderophore as well as by their concentrations and kinetics of exchange, while the latter is governed by the existence of an uptake mechanism for, and its affinity to, the Fe complex.

The data presented in this article support the hypothesis that some pseudobactin siderophores are intrinsically less available to rhizosphere microfloras than various hydroxamate siderophores. The faster uptake rates of Fe-pseudobactin complex by pseudobactin-excreting pseudomonad strains and their ability to utilize a large variety of exogenous siderophores are certainly important parameters in reducing the availability of Fe to other microorganisms in the rhizosphere.

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