APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1992, p. 630–635
0099-2240/92/020630-06$02.00/0

Vol. 58, No. 2

# Characterization of Fluorescent Siderophore-Mediated Iron Uptake in *Pseudomonas* sp. Strain M114: Evidence for the Existence of an Additional Ferric Siderophore Receptor

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Received 13 August 1991/Accepted 19 November 1991

**In *Pseudomonas* sp. strain M114, the outer membrane receptor for ferric pseudobactin M114 was shown to transport ferric pseudobactins B10 and A225, in addition to its own. The gene encoding this receptor, which was previously cloned on pCUP3, was localized by Tn*5* mutagenesis to a region comprising >1.6 kb of M114 DNA. A mutant (strain M114R1) lacking this receptor was then created by a marker exchange technique. Characterization of this mutant by using purified pseudobactin M114 in radiolabeled ferric iron uptake studies confirmed that it was completely unable to utilize this siderophore for acquisition of iron. In addition, it lacked an outer membrane protein band of 89 kDa when subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. As a result, growth of the mutant was severely restricted under low-iron conditions. However, this phenotype was reversed in the presence of another fluorescent siderophore (pseudobactin MT3A) from *Pseudomonas* sp. strain MT3A, suggesting the presence of a second receptor in strain M114. Furthermore, wild-type *Pseudomonas* sp. strain B24 was not able to utilize ferric pseudobactin MT3A, and this phenotype was not reversed upon expression of the M114 receptor encoded on pCUP3. However, a cosmid clone (pMS1047) that enabled strain B24 to utilize ferric pseudobactin MT3A was isolated from an M114 gene bank. Radiolabel transport assays with purified pseudobactin MT3A confirmed this event. Plasmid pMS1047 was shown to encode an outer membrane protein of 81 kDa in strain B24 under iron-limiting conditions; this protein corresponds to a similar protein in strain M114.**

An important factor in the biological control of plant diseases by beneficial *Pseudomonas* spp. is the production of iron-chelating ligands (siderophores). The reduction in the availability of iron in the rhizosphere due to these fluorescent siderophores is suggested to result in the suppression of potential plant-deleterious microorganisms (11, 12). The structures of some fluorescent siderophores from both plant-beneficial and -deleterious strains have been determined. The siderophores usually consist of a fluorescent chromophore linked to a peptide chain and differ mainly in the size and composition of the peptide chain (6, 25, 30, 31). The uptake of these complexes has been shown to be mediated by specific receptors in the outer membrane (14, 16, 22). The production of both biosynthetic (17, 19) and uptake (9, 14, 23) proteins is stringently regulated by iron. It was recently shown (23) that this is mediated via a negative repressor analogous to the *Escherichia coli* Fur (ferric uptake regulation) protein. However, recent evidence indicates that a transcriptional activator is required for the expression of a pseudomonad iron-regulated gene, indicating that the siderophore uptake system may be regulated both positively and negatively (24).

The ability of any *Pseudomonas* strain to utilize a given ferric siderophore complex depends on that strain possessing a receptor specific for the ferric siderophore complex. In addition to the outer membrane receptors, other less specific proteins are also involved in the transport of siderophores. It has previously been shown in *Pseudomonas* sp. strain M114 that the ferric siderophore complex (ferric pseudobactin M114) is transported across the membrane into the cytosol before the release of iron (22). However, very little is known about how the complex crosses the cell envelope. Sequence analysis of an outer membrane receptor protein from *Pseudomonas putida* WCS358 showed regions with homology to TonB-dependent ferric siderophore receptor proteins of *E. coli* (3), suggesting the presence of a TonB-like protein in *P. putida*. The TonB protein is required for the active transport of ferric siderophores and vitamin $B_{12}$ across the outer membrane in *E. coli* by energizing the outer membrane (26). The potential exists to improve the iron uptake capacity of a beneficial *Pseudomonas* strain through the introduction of extra ferric siderophore receptors into the strain. These extra receptors may utilize the existing siderophore transport machinery within the cell, and the system would also allow a strain to utilize a wider diversity of ferric siderophore complexes for its iron acquisition in an iron limited environment.

Under iron-limiting conditions, four proteins are produced in the outer membrane of *Pseudomonas* sp. strain M114; these proteins are not present in conditions of excess iron (23). One of these proteins (89 kDa) is required for the transport of ferric pseudobactin M114 (22). In this paper we report the construction and characterization of a mutant of strain M114 lacking this ferric siderophore receptor. In addition, we demonstrate the existence of another M114 receptor that is required for the uptake of a ferric siderophore not derived from strain M114.

## MATERIALS AND METHODS

**Strains, plasmids, and culture conditions.** The bacterial cultures and plasmids used are listed in Table 1. *Pseudomo-*

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TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Relevant characteristics[a] | Source or reference |
|---|---|---|
| *Pseudomonas* sp. strains | | |
| M114 | Wild type, $Ap^r$ $Flu^+$ | 21 |
| M114R1 | $Ap^r$ $Km^r$, Fe pseudobactin M114 receptor mutant | This study |
| B24 | Wild type, $Ap^r$ | 22 |
| MT3A | Wild type, $Ap^r$ | University College, Cork culture collection |
| *Escherichia coli* | | |
| HB101 | *recA hsdB hsdM strA pro leu thi* | 4 |
| HB101::Tn5 | $Km^r$ | Utrecht University |
| C2110 | $Nal^r$ $Rif^r$, *polA1 rha his* | Utrecht University |
| LE392 | *lacY1 galK2 galT22* | 15 |
| Plasmids | | |
| pCUP3 | $Tc^r$ $Tra^-$ IncP Sid $U^{+b}$ | 22 |
| pPH1JI | $Gm^r$ $Sp^r$ IncP | 1 |
| pMS1047 | $Tc^r$ $Mob^+$ $Tra^-$ IncP Sid $U^{+b}$ | This study |
| pMS639 | $Tc^r$ $Mob^+$ $Tra^-$ IncP Sid $B^{+c}$ Sid $U^{+b}$ Sid $R^{+d}$ | 23 |

[a] Abbreviations for drug resistance: Ap, ampicillin; Gm, gentamicin; Km, kanamycin; Nal, nalidixic acid; Rif, rifampin; Sp, spectinomycin; Tc, tetracycline; Tp, trimethoprim.
[b] $U^+$, ferric siderophore uptake functions from strain M114 are present.
[c] $B^+$, siderophore biosynthesis functions from strain M114 are present.
[d] $R^+$, siderophore regulatory functions from strain M114 are present.

*nas* cultures were grown at 28°C on minimal sucrose-asparagine (SA) medium (28), chrome azurol S medium (29), and King B (KB) medium (10). For siderophore cross-feeding studies, iron-limited conditions were obtained by the addition of ethylenediamine di(*o*-hydroxyphenyl) acetic acid at concentrations up to 0.1 mM. $FeCl_3$ was added at 75 μM for iron-sufficient conditions. *E. coli* strains were cultured at 37°C on Luria-Bertani (LB) medium (15). Kanamycin (50 μg/ml for *E. coli*, 25 μg/ml for *Pseudomonas* sp.), tetracycline (25 μg/ml for *E. coli*, 70 μg/ml for *Pseudomonas* sp.), nalidixic acid (15 μg/ml for *E. coli*), gentamicin (5 μg/ml for *Pseudomonas* sp.), and ampicillin (50 μg/ml for *E. coli*) were used as required.

**DNA isolation and genetic manipulations.** Plasmids were isolated by using the rapid procedure of Birnboim and Doly (2). Large-scale preparations involved equilibrium centrifugation in a CsCl gradient containing ethidium bromide. Chromosomal DNA isolation was carried out by the method of Meade et al. (18). DNA was digested as recommended by the restriction endonuclease manufacturer (Boehringer Mannheim Biochemicals, Germany). Southern blots and hybridizations were performed with Gene Screen Plus hybridization transfer membranes as recommended by the manufacturer (Biotechnology Systems, NEN Research Products, Boston). DNA probes were labeled with α-$^{35}$S-dATP by using the Prime-a-Gene random labeling system in accordance with the recommendations of the manufacturer (Amersham International plc., United Kingdom). Labeled DNA was selectively precipitated with ammonium acetate and ethanol. Plasmids were introduced into *E. coli* by transformation with the calcium chloride procedure (7). Conjugations of plasmids into *Pseudomonas* sp. were carried out as described previously (22). All other genetic manipulations were carried out as described by Maniatis et al. (15).

**Tn*5* mutagenesis of pCUP3.** Plasmid pCUP3 was transformed into HB101::Tn*5*, and the transformants were pooled and mated en masse into *E. coli* C2110 as described previously (13). $Nal^r$, $Tc^r$, and $Km^r$ exconjugants were pooled; plasmid DNA was then isolated and transformed into HB101. Transformants were mated en masse into strain B24, and individual exconjugants were screened for the inability to utilize ferric pseudobactin M114 as a source of iron by streaking on KB plates containing 20 μM pseudobactin M114 and 100 μM bipyridyl.

**Marker exchange procedure.** Tn*5* containing pCUP3 clones with an inactivated receptor gene were used to create a mutated receptor gene on the M114 chromosome by using a marker exchange procedure (27) with minor modifications (8). Candidate clones were transformed into HB101 and then mated into strain M114 in a triparental mating. Exconjugants were then mated with HB101(pPH1JI), which is of the same incompatibility group as pCUP3. Potential marker exchanged mutants were selected on LB plates containing gentamicin, ampicillin, and kanamycin. All candidates were tested for loss of $Tc^r$, i.e., loss of vector sequences, by plating on the above plates containing tetracycline.

**Siderophore cross-feeding assays and outer membrane preparations.** Cross-feeding tests, outer membrane preparations, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were carried out as described previously (22).

**Siderophore purification.** Siderophores were harvested from 24-h, 1-liter cultures grown in SA medium at 28°C under vigorous aeration. The pH was maintained at 7.0 to 7.3 by buffering the growth medium with 2% (wt/vol) morpholine propanesulfonic acid. Cells were removed by centrifugation, and crude siderophore extracts were prepared by the method of Yang and Leong (31) with minor modifications. Culture supernatant fluids were concentrated to 100 ml at 28°C in a Buchi Rotavapor apparatus, and the concentrates were saturated with ammonium sulfate. The resulting solutions were extracted with phenol-chloroform (1:1, wt/vol). Organic phases were centrifuged and filtered before dilution with excess diethyl ether. Small volumes of distilled deionized water were used to extract the crude siderophore preparation, which was then freeze-dried. The crude siderophore was further purified by the method of J. Glennon et al. (9a). Extracts were dissolved in distilled deionized water and passed through a polymeric column (containing 8-μm polystyrene divinylbenzene particles; Polymer Laboratories) of a metal-free high-performance liquid chromatography system. The system was equilibrated with 15 mM acetate buffer (pH 6.0) and 7% acetonitrile. Fractions were detected at 405 nm with a Waters Lambda-Max model 481 LC spectrophotometer, and single peaks were collected and freeze-dried. The resulting powder was dissolved in 0.02 M pyridine acetate buffer (pH 6.0), passed through a Bio-Gel P-2 column equilibrated with the same buffer, and then freeze-dried. The powder was stored at −20°C until use.

**Transport assays.** A labeled iron stock was prepared by mixing $^{55}FeCl_3$ (78 mCi/mmol) with unlabeled $FeCl_3$ in a ratio of 1:3 to give a stock concentration of 10 mM. This stock solution was mixed with excess purified siderophore, previously dissolved in 0.06 M sodium-potassium phosphate buffer (pH 7.4), to prepare the labeled ferric siderophore complex (specific activity, 20 μCi/mmol of $Fe^{3+}$). Transport assays were performed as described by Magazin et al. (14)

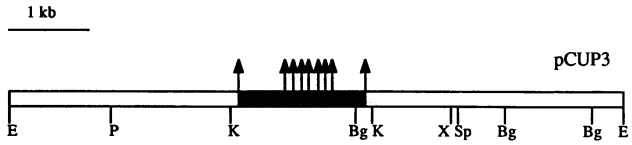


FIG. 1. Restriction map of the 7.8-kb *Eco*RI fragment of pCUP3. The shaded area represents the area spanned by Tn*5* insertions (arrows) that inactivate the receptor gene. Abbreviations: Bg, *Bgl*II; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; Sp, *Sph*I; X, *Xho*I.

with minor modifications. For transport assays, cultures were grown in 50-ml volumes of SA containing appropriate concentrations of antibiotics at 28°C for 12 h in 250-ml Erlenmeyer flasks with vigorous shaking. Cells were harvested by centrifugation, washed twice with SA at 28°C, and resuspended to an optical density of 0.2 at 650 nm. Cultures were dispensed in 20-ml volumes into 250-ml Erlenmeyer flasks and incubated for 0.5 h at 28°C; then labeled ferric siderophore to a final concentration of 1 μM was added. After the labeled compound was added, 0.5-ml aliquots of cells were removed at the time intervals indicated and filtered with a Millipore manifold. Cells were washed twice with 15-ml volumes of SA medium. Washed filters were transferred to 10 ml of scintillation fluid (EcoLite, ICN Biomedicals Inc., Irvine, Calif.), and radioactivity was counted by using the tritium channel of a Beckman LS700 liquid scintillation counter.

## RESULTS

**Localization of the gene encoding the ferric pseudobactin M114 outer membrane receptor.** The ferric pseudobactin M114 outer membrane receptor gene was recently identified on a M114 gene bank cosmid clone (pMS639) and subcloned to a 7.8-kb *Eco*RI fragment in pCUP3 (22). In the present study pCUP3 was saturated with Tn*5*, and insertions resulting in inactivation of receptor functions were further characterized. Receptor functions were evaluated as described previously with *Pseudomonas* sp. strain B24, which is unable to utilize pseudobactin M114 as a source of ferric iron (22). In this system the receptor functions encoded on pCUP3 enable strain B24 to utilize pseudobactin M114, but when the receptor functions are inactivated this phenotype is reversed. Tn*5* insertions on receptor-deficient clones were mapped at nine unique sites spanning a region of 1.6 kb (Fig. 1).

**Chromosomal mutation of the ferric pseudobactin M114 receptor gene.** Tn*5* mutations of the receptor gene on pCUP3 were used in a marker exchange procedure to obtain ferric pseudobactin M114 receptor mutants in the parent strain, M114. Clones were initially screened on chrome azurol S plates for overproduction of siderophore and a small colony phenotype. On this medium, siderophore production is indicated by an orange halo surrounding the colony. Whereas wild-type clones produce a small defined halo, a large halo surrounding a small colony is indicative of ferric siderophore uptake or regulatory mutants (29). All clones exhibited the same mutant phenotype on chrome azurol S plates; one of these (strain M114R1) was randomly chosen for further analysis.

**Confirmation of the marker exchange event.** The site-specific exchange of the mutated receptor gene from pCUP3 with the receptor gene on the chromosome was confirmed by DNA hybridization. Southern blots of *Eco*RI restriction digests of chromosomal DNA from wild-type strain M114 and putative receptor mutant strain M114R1 were probed with the 1.8-kb *Kpn*I fragment of pCUP3, within which all of the receptor-inactivating Tn*5* insertions are located (Fig. 1). The fragment containing the ferric pseudobactin M114 receptor gene increased from 7.8 kb in the wild-type chromosome to 13.5 kb in the M114R1 chromosome, which is consistent with the addition of a Tn*5* insertion (data not shown).

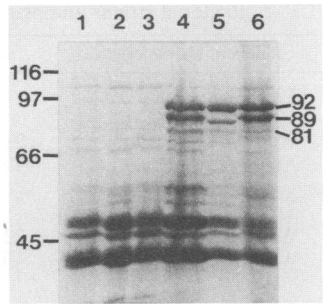


FIG. 2. Outer membrane protein profiles on a 9% SDS–polyacrylamide gel. Cells of strains M114 (lanes 1 and 4), M114R1 (lanes 2 and 5), and M114R1(pCUP3) (lanes 3 and 6) were grown under excess-iron (lanes 1, 2, and 3) and iron-limited (lanes 4, 5, and 6) conditions. The positions of molecular mass standards (on the left) are listed in kilodaltons.

**Characterization of the receptor mutant.** It was previously reported that the putative receptor gene on the 7.8-kb *Eco*RI fragment encodes a protein of 89 kDa in the outer membrane of strain M114 (22). It would therefore be expected that a ferric pseudobactin M114 receptor mutant would lack an outer membrane protein of this size. This was found to be the case in strain M114R1, in which an 89-kDa protein band was missing from the outer membrane profile on SDS-PAGE (Fig. 2). The other proteins expressed in the outer membrane of strain M114 under iron-limited conditions (92, 88, and 81 kDa) (23) were still present in the mutant strain M114R1.

Physiological characterization of mutant strain M114R1 indicated that it had a reduced ability to grow under low-iron conditions when compared with the parent strain M114. This is consistent with a defect in the ferric siderophore uptake system. Further substantiation of this was obtained by using siderophore cross-feeding assays as described previously (22). In these assays strain M114R1 was unable to utilize the siderophore of the parent strain (data not shown). When the receptor-containing plasmid pCUP3 was introduced into strain M114R1, this phenotype was reversed, allowing strain M114R1(pCUP3) to utilize pseudobactin M114. Furthermore, the phenotype of the complemented mutant was the same as that of the wild-type strain M114 grown on chrome azurol S plates. In addition, the outer membrane protein profiles of strain M114R1(pCUP3) indicated that the 89-kDa protein band, which was missing in strain M114R1, was restored by plasmid pCUP3 (Fig. 2).

**Uptake studies with purified siderophore.** Transport assays with $^{55}Fe^{3+}$-labeled pseudobactin M114 indicated that wild-type cells accumulated $^{55}Fe$ within 1 min and were almost totally saturated within 30 min (Fig. 3). In contrast, receptor mutant strain M114R1 was totally deficient in its accumulation of $^{55}Fe$, even after 1 h. As expected, this phenotype was reversed by pCUP3, which is consistent with this plasmid harboring the gene coding for the outer membrane receptor for ferric pseudobactin M114 (Fig. 3).

The ability to use pseudobactins of other wild-type *Pseu-*

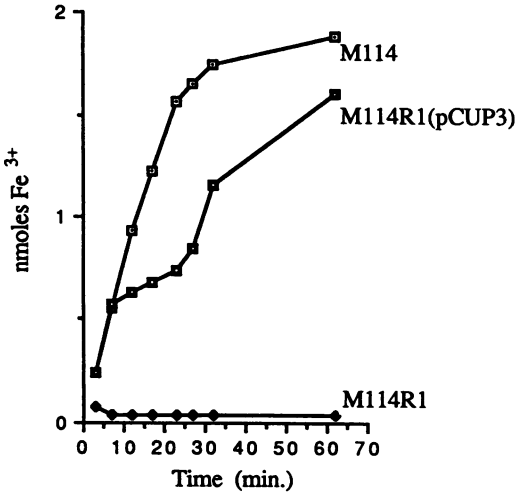


FIG. 3. Transport of $^{55}Fe^{3+}$-labeled ferric pseudobactin M114. Values are expressed as nanomoles of $Fe^{3+}$ transported at a cell density (optical density at 650 nm) of 0.2 (~$1.2 \times 10^8$ CFU/ml).

*domonas* strains was tested on low-iron plates after the test pseudobactins were added. Neither strain M114 nor M114R1 utilized pseudobactin WCS358, WCS374, WCS366, WCS361, BN7, BN8, BN2, A124, 7SR1, or PAO. Strain M114 was able to utilize pseudobactins M114, B10, A225, and MT3A to reverse the iron starvation conditions induced by ethylenediamine di(*o*-hydroxyphenyl) acetic acid. However, the receptor mutant strain M114R1 was not capable of using pseudobactin M114, B10, or A225, indicating that the receptor involved in the transport of these ferric pseudobactin complexes is the ferric pseudobactin M114 receptor.

**Evidence for an additional ferric pseudobactin receptor in strain M114.** Although the receptor mutant (strain M114R1) was unable to use M114, B10, or A225 pseudobactin, cross-feeding assays indicated that it was still capable of utilizing pseudobactin from strain MT3A for its iron acquisition. This may point to the presence of another ferric pseudobactin receptor in strain M114, a receptor that is not involved in the transport of ferric pseudobactin M114 but is required for the transport of at least one other ferric pseudobactin complex.

**Cloning of a second receptor gene in strain M114.** Genetic evidence for the presence of this extra receptor was obtained by cloning a strain M114 gene that encoded the uptake of ferric pseudobactin MT3A. Cross-feeding tests indicated that strain B24 could not utilize pseudobactin MT3A, thus providing a convenient selection procedure. A pLAFR1-based M114 gene bank was mated into strain B24, and a clone was selected that enabled cross feeding by pseudobactin MT3A to occur. This clone (pMS1047) contained an insert of ~21 kb, and restriction endonuclease analysis indicated that it did not overlap any of the cloned siderophore-related genes from strain M114 that were outlined previously (22).

**Biochemical characterization of the second receptor.** The outer membrane protein profile of strain B24(pMS1047) showed the presence of a protein band not normally present in strain B24 (Fig. 4). This corresponds with a protein of 81 kDa in the outer membrane of strain M114. Under conditions of excess iron this protein is not produced by strain B24(pMS1047), as is the case with the 81-kDa protein in strain M114. This indicates that the iron-regulated promoter region of the gene involved is also present and functional in

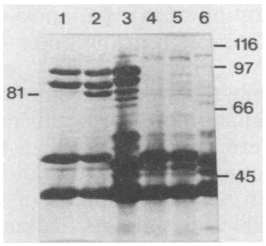


FIG. 4. Outer membrane protein profiles on a 9% SDS–polyacrylamide gel. Cells of strains B24 (lanes 1 and 4), B24(pMS1047) (lanes 2 and 5), and M114 (lanes 3 and 6) were grown under iron-limited (lanes 1, 2, and 3) and excess-iron (lanes 4, 5, and 6) conditions. The positions of molecular mass standards (on the right) are listed in kilodaltons.

pMS1047. To verify that the cloned gene on pMS1047 encoded a receptor for ferric pseudobactin MT3A, it was necessary to isolate this pseudobactin for characterization of the transport properties of the receptor. Transport assays with $^{55}Fe^{3+}$ pseudobactin MT3A confirmed that strain B24 was unable to accumulate this ferric pseudobactin complex (Fig. 5). However, after pMS1047 was introduced into this strain, $^{55}Fe$ accumulated at a rate comparable to that in strain M114 (Fig. 5). We can therefore conclude that pMS1047 encodes an outer membrane receptor protein from strain M114 that is required for the uptake of ferric pseudobactin MT3A.

## DISCUSSION

The ferric pseudobactin M114 receptor gene, which was previously cloned (22), has now been localized by using Tn*5* mutagenesis to a region spanning at least 1.6 kb of DNA on pCUP3, flanked on both sides by *Kpn*I restriction sites. The complete receptor gene, however, is probably larger than the 1.6-kb fragment for the following reasons. (i) The receptor protein encoded on pCUP3 is 89 kDa (as estimated by SDS-PAGE), therefore requiring more than 1.6 kb of DNA. (ii) The *Kpn*I fragment of 1.8 kb has been cloned into pMP220 in both orientations and shown not to contain a promoter region (data not shown). A mutant (strain M114R1) lacking the ferric pseudobactin M114 receptor was obtained by using a pCUP3::Tn*5* clone in a marker exchange proce-

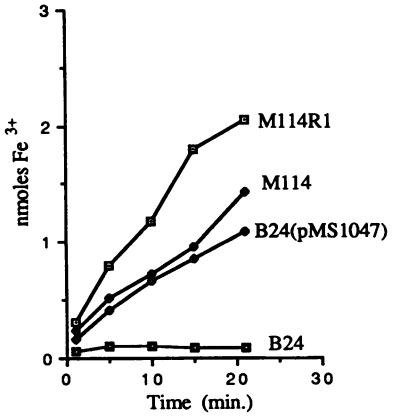


FIG. 5. Transport of $^{55}Fe^{3+}$-labeled ferric pseudobactin MT3A. Values are expressed as nanomoles of $Fe^{3+}$ transported at a cell density (optical density at 650 nm) 0.2 (~$1.2 \times 10^8$ CFU/ml).

dure. Characterization of this mutant confirmed that it was missing an 89-kDa protein in the outer membrane and was also incapable of accumulating $^{55}Fe^{3+}$ pseudobactin M114. Even after extended periods of time we were unable to detect any accumulation of $^{55}Fe$ by this mutant; these results indicate a complete inability to utilize ferric pseudobactin M114. This phenotype is similar to that of *Pseudomonas* sp. strain B10 (14), in which no uptake of ferric-pseudobactin B10 was detectable in mutants lacking its receptor. In contrast, however, a mutant of *P. putida* WCS358 that lacked the 86-kDa outer membrane receptor for ferric pseudobactin 358 retained a residual uptake capacity of ~30% (3). This may suggest the presence in this strain of another outer membrane receptor with a broader spectrum specificity. It seems likely from the results obtained in this study that the 89-kDa protein, missing from the outer membrane of mutant strain M114R1, is the only receptor in the outer membrane of strain M114 that is responsible for transporting ferric pseudobactin M114.

The ferric pseudobactin M114 receptor may not be solely specific for ferric pseudobactin M114. By using purified pseudobactins, it was shown that this receptor was capable of transporting ferric pseudobactins B10 and A225 in addition to its own. However, recent chemical analysis indicates that pseudobactin M114 is very similar, if not identical, to pseudobactin B10 (9a). Since strain A225 cannot utilize ferric pseudobactin B10 (14), its siderophore (pseudobactin A225) is intrinsically different from pseudobactin B10. This strongly indicates that the ferric pseudobactin M114 receptor is capable of transporting more than one ferric pseudobactin complex. Although this may be the case, our evidence indicates that this is not a very broad-spectrum receptor because it was incapable of transporting iron complexed to 10 other characterized pseudobactinlike siderophores. However, the presence of another receptor in strain M114 improves the broad-spectrum capability of the strain for uptake of different pseudobactins. The presence of broad-spectrum and/or multiple ferric siderophore receptors in a strain may be an important factor for survival and competition in the rhizosphere. By increasing the spectrum of siderophores that a strain can utilize, through the introduction of extra receptors, it may be possible to improve the performance of strains currently involved in biological control. Outer membrane receptors have been successfully introduced into strains A124, A225 (14), WCS374 (16), and B24 (22; this study), increasing the range of siderophores that these strains may utilize in siderophore antibiosis (14, 16) and cross-feeding assays (22; this study). These receptors presumably utilize the machinery already present in these strains for transport across the periplasm and cytoplasmic membrane and for release of the iron from the ferric siderophore complex (22). It would be interesting to determine the performance of these engineered strains in the rhizosphere to evaluate this concept.

The gene encoding the transport of ferric pseudobactin MT3A, which was cloned on pMS1047, was shown to allow strain B24 to utilize this ferric pseudobactin as efficiently as did wild-type strain M114 (Fig. 5). It is evident, however, that mutant strain M114R1 transports more ferric pseudobactin MT3A than its parent does. This probably reflects the extreme iron-starved state of these cells compared with wild-type cells before the addition of labeled ferric pseudobactin MT3A. The iron-regulated protein encoded on pMS1047 is probably the 81-kDa protein from the outer membrane of strain M114. This receptor is required for the recognition and initial stage of transport of ferric pseudobactin MT3A. In addition to this 81-kDa protein and the 89-kDa ferric pseudobactin M114 receptor, there are two other iron-regulated protein bands (92 and 88 kDa) evident in the outer membrane profile of strain M114 (23). The function of these proteins has not been determined; however, since their molecular weights are compatible with those of ferric pseudobactin receptors, it is conceivable that they function as additional receptors in this strain.

Inactivation of the ferric pseudobactin M114 receptor greatly retarded the growth of the resulting mutant. Transport assays confirmed that it could no longer bind its own ferric pseudobactin complex (Fig. 3). As a result, the presence of its own siderophore (pseudobactin M114) reduced the available iron in growth media, greatly impeding its growth. A similar effect is seen in wild-type strains when they are grown in the presence of ferric siderophores that they cannot use. This suggests the intriguing proposition that cells unable to use different siderophores in the rhizosphere could be placed at a disadvantage and thus may provide an evolutionary pressure for acquiring other receptors by natural selection. Since we have now demonstrated the existence of a receptor in strain M114 for the uptake of iron complexed to a siderophore (pseudobactin MT3A) that it does not produce, this may indeed be the case. The situation with strain M114 in this context would appear to be similar to the observation that *E. coli* has outer membrane receptors for the transport of fungal hydroxymate siderophores, not produced by *E. coli* itself (5, 20). This would therefore suggest that engineering strains by incorporating multiple receptors has potential important implications in biocontrol.

## ACKNOWLEDGMENTS

We thank Pat Higgins for technical assistance and everyone who supplied strains and plasmids for this work.

This work was supported in part by grants from the Irish Science and Technology Agency (ST/B10/335/88 and ST/008/89) and the European Community (BAP-0413 C [EDB], FLAIR-AGRF-CT91-0049 [DTEE], and ECLAIR-AGRE-0019-C).

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