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Pseudomonas fluorescens Pirates both Ferrioxamine and Ferricoelichelin Siderophores from *Streptomyces ambofaciens*

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Iron is essential in many biological processes. However, its bioavailability is reduced in aerobic environments, such as soil. To overcome this limitation, microorganisms have developed different strategies, such as iron chelation by siderophores. Some bacteria have even gained the ability to detect and utilize xenosiderophores, i.e., siderophores produced by other organisms. We illustrate an example of such an interaction between two soil bacteria, *Pseudomonas fluorescens* strain BBc6R8 and *Streptomyces ambofaciens* ATCC 23877, which produce the siderophores pyoverdine and enantiopyochelin and the siderophores desferrioxamines B and E and coelichelin, respectively. During pairwise cultures on iron-limiting agar medium, no induction of siderophore synthesis by *P. fluorescens* BBc6R8 was observed in the presence of *S. ambofaciens* ATCC 23877. Cocultures with a *Streptomyces* mutant strain that produced either coelichelin or desferrioxamines, as well as culture in a medium supplemented with desferrioxamine B, resulted in the absence of pyoverdine production; however, culture with a double mutant deficient in desferrioxamines and coelichelin production did not. This strongly suggests that *P. fluorescens* BBbc6R8 utilizes the ferrioxamines and ferricoelichelin produced by *S. ambofaciens* as xenosiderophores and therefore no longer activates the production of its own siderophores. A screening of a library of *P. fluorescens* BBc6R8 mutants highlighted the involvement of the TonB-dependent receptor FoxA in this process: the expression of *foxA* and genes involved in the regulation of its biosynthesis was induced in the presence of *S. ambofaciens*. In a competitive environment, such as soil, siderophore piracy could well be one of the driving forces that determine the outcome of microbial competition.

Bacteria detect, assimilate, and integrate different environmental signals in order to better adapt to their habitat and cope with changes in environmental conditions. Multiple signaling pathways allow them to communicate with each other within the same species or between different species (1). This can be achieved through the production and detection of diffusible molecules in the environment. In response to these interactions, the microorganisms have developed complex metabolic and physiological responses. One of the essential environmental factors vital for organisms is iron. It plays an essential role in many biological processes, such as DNA synthesis, respiration, and photosynthesis. Iron can adopt two different ionic forms, Fe^{2+} and Fe^{3+}. This property makes it an important player in the oxidation-reduction reactions in the cell. However, while iron is an abundant element on earth, its bioavailability is reduced in aerobic environments, such as soil. Ferric iron (Fe^{3+}) forms insoluble ferric hydroxides (with a solubility product of $\sim 10^{-39}$) in the presence of oxygen (2–4). Therefore, iron is a limiting factor for the growth of microorganisms.

To overcome the limitation of iron bioavailability, aerobic bacteria have developed several highly specialized strategies to acquire the metal from different sources. One of them consists of producing siderophores under conditions of iron deficiency (5). Siderophores are low-molecular-mass molecules (200 to 2,000 Da) with diverse iron affinities, and to date, more than 500 different chemical structures of siderophores have been identified. A single bacterial species can produce different siderophores, although in general, not all are produced at the same time. For example, *Pseudomonas aeruginosa* is able to switch between the synthesis of its siderophores pyoverdine and pyochelin depending on environmental conditions. In a severe iron depletion environment, pyoverdine, the most effective but metabolically expensive siderophore, is produced. However, in environments moderately depleted of iron, pyochelin, a less metabolically expensive siderophore, is used to take up iron (6).

In addition to acquiring iron via specific receptors for their own siderophores, many bacteria possess uptake systems for xenosiderophores, i.e., siderophores produced by other organisms. In competitive environments, like soil, this allows them to utilize exogenous siderophores in a strategy known as siderophore piracy (7, 8). A study published by Cornelis and Bodilis (9) revealed that the majority of siderophore receptors are conserved in the different representatives of a species (the core receptor), while others are acquired by horizontal gene transfer. For example, *Pseudomonas fluorescens* SBW25 possesses 23 other putative siderophore receptors in addition to the receptor of its own pyoverdine. This allows the bacterium to take up 19 heterologous pyoverdines from

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TABLE 1 Bacterial strains used in this work

Strain	Characteristics	Reference
S. ambofaciens ATCC 23877	Reference strain (wild type)	18
S. coelicolor M512	Δ*redD* Δ*actII*-ORF4 from *S. coelicolor* M145; deficient in undecylprodigiosin and actinorhodin production	40
S. coelicolor M512 *desD*	Derived from M512; deficient in desferrioxamine production; *S. coelicolor* M512 *desD*::Tn*5062*	This study
S. coelicolor M512 *cchH*	Derived from M512; deficient in coelichelin production; *S. coelicolor* M512 *cchH*::Tn*5062*	This study
S. coelicolor Δ*des* Δ*cch*	Derived from M145; deficient in desferrioxamine and coelichelin production [*desEFABCD*:: *aac*(*3*)*IV*-*cchABCDEFGHIJK*::*vph*; strain W13)	37
E. coli ET12567(pUZ8002)	Donor strain in intergeneric conjugation; a methylation-defective strain	31
P. fluorescens BBc6R8	Isolated from *Laccaria bicolor*	19
P. fluorescens SBW25	Isolated from rhizosphere of sugar beet	67
P. fluorescens Pf0-1	Isolated from agricultural soil	68
P. aeruginosa PAO1	Human opportunistic pathogen	69
P. fluorescens P28H6	Mutant derived from BBc6R8, Tn*5* insertion in *foxA*	A. Deveau, unpublished data
P. fluorescens P18B10	Mutant derived from BBc6R8, Tn*5* insertion in *foxA*	Deveau, unpublished
Library of 60 *P. fluorescens* strains	Strains isolated from the bulk soil of a forest nursery, the ectomycorrhizosphere, and the ectomycorrhizas of *L. bicolor*/Douglas fir	70

25 different *Pseudomonas* isolates (10). *Pseudomonas fragi*, which does not produce siderophores, is able to use enterobactin, pyoverdine, and desferrioxamine B, produced by the bacterial species *Escherichia coli*, *P. fluorescens*, or *P. aeruginosa* and *Pseudomonas stutzeri*, respectively (11). Other genera of bacteria, like *Yersinia* (12), *Erwinia* (13), *Vibrio* (14), and *Amycolatopsis* (15), are also able to detect and to take up xenosiderophores, in addition to the use of their own siderophores. The wide distribution of xenosiderophore uptake genes in many bacterial species suggests that siderophore piracy is a common process in multispecies communities. However, most of these studies were performed *in vitro* using purified siderophores, and little is known regarding the occurrence of siderophore piracy during biotic interactions.

Actinomycetes and pseudomonads represent two of the major groups of bacteria found in soils and rhizospheres (16, 17) and are likely to simultaneously utilize similar resources, such as iron, or even to compete for them. Both groups produce siderophores to take up this essential element, but different types of molecules are produced by the two groups. Many pseudomonads produce pyoverdines, a family of high-affinity catecholate-hydroxamate siderophores, while the actinomycetes of the genus *Streptomyces*, for instance, secrete hydroxamate siderophores, such as desferrioxamines and coelichelin. In this study, we show, through a pairwise interaction between the two soil bacteria *Streptomyces ambofaciens* ATCC 23877 (18) and *P. fluorescens* BBc6R8 (R8) (19, 20), that the *P. fluorescens* strain uses the streptomycete's siderophores and does not induce the production of its own siderophores, pyoverdine and enantiopyochelin, in the presence of *S. ambofaciens* ATCC 23877 when grown under iron-limiting conditions. Our study reveals that *P. fluorescens* recognizes *Streptomyces* siderophores through FoxA, a TonB-dependent receptor whose biosynthesis is induced in the presence of *S. ambofaciens* ATCC 23877 siderophores.

MATERIALS AND METHODS

Bacterial strains, media, and culture conditions. All *Streptomyces*, *Pseudomonas*, and *E. coli* strains used in this work are listed in Table 1. The *Streptomyces* strains were manipulated as described by Kieser et al. (21). *Pseudomonas* cell stocks were prepared by streaking each strain on Trypticase soy agar (TSA) medium (containing kanamycin at 20 μg/ml for the P18B10 and P28H6 mutants) and incubating the plates for 48 h at 26.5°C or at 37°C for *P. aeruginosa* PAO1. A single clone was resuspended in 50 μl water, spread on TSA medium, and then grown for 48 h. Cells were collected and centrifuged at 13,500 × *g* for 2 min, and the pellet was washed twice with sterile water. The pellet was then resuspended in sterile water to an optical density at 600 nm (OD_{600}) of 0.7 (10^9 CFU per ml).

To analyze the effect of *S. ambofaciens* strain ATCC 23877 on the production of siderophores by *P. fluorescens* BBc6R8, a bioassay was set up on 26A agar medium (for 400 ml, 0.4 g glucose, 6 g tryptone, 2 g NaCl, pH 7.2). *Streptomyces* and *Pseudomonas* were streaked side by side 3 mm from each other and were incubated at 26.5°C (37°C with *P. aeruginosa* PAO1) for 2 days. The streaks were made up of 2 μl of a spore suspension of *S. ambofaciens* ATCC 23877 at 10^9 CFU/ml and 2 μl of *Pseudomonas* at 10^7 CFU/ml. A control experiment with *Pseudomonas* alone was done for each incubation condition. When indicated, the iron chelator 2,2′-bipyridyl (200 μM), desferrioxamine B mesylate (200 μM), or $FeSO_4 \cdot 7H_2O$ (12.5 μM) was added to the 26A agar medium. For fluorescence detection, a Chemidoc XRS (Bio-Rad) was used with UV transmission (302 nm).

To test the ability of the *S. coelicolor* Δ*des* Δ*cch* mutant (strain W13) to take up exogenous siderophores, two different experiments were carried out. The first experiment was as follows. One hundred microliters of a spore suspension of *S. coelicolor* M512 at 2 × 10^8 CFU/ml or 100 μl of a cell suspension of *P. fluorescens* BBc6R8 at 2 × 10^7 CFU/ml was spread on a 26A agar plate supplemented or not with 2,2′-bipyridyl at 100 or 200 μM. After 3 days of growth at 26.5°C, plugs (8 mm in diameter) were obtained from confluent regions and placed on 26A agar plates containing 200 μM 2,2′-bipyridyl with spores of strain W13 (10^6 or 10^9 CFU) evenly spread on them. The plates were incubated at 26.5°C for 3 days. The halo of growth around the plugs was then analyzed. In the second experiment, strains M512 and BBc6R8 (10^4 CFU) were streaked on both side of a 26A agar plate supplemented with 200 μM 2,2′-bipyridyl. The plates were incubated at 26.5°C for 3 days, and the W13 mutant (2 μl at 10^9 CFU/ml) was then streaked at about 3 mm alongside strain M512 or BBc6R8.

DNA manipulation and transcriptional analysis. Isolation, cloning, and manipulation of DNA were carried out as previously described for *Streptomyces* (22, 23), *Pseudomonas* (24), and *E. coli* (25). Amplification of DNA fragments by PCR was performed with Dreamtaq DNA polymerase (Fermentas). All primers are described in Table 2. The transposon insertion site in *P. fluorescens* BBc6R8 was determined using a double-round

TABLE 2 Primer pairs used in qPCR analyses or to determine transposon insertion sites in the *P. fluorescens* BBc6R8 genome

Name	Sequence (5′–3′)	Studied gene
hrdB-F	CGCGGCATGCTCTTCCT	*hrdB*
hrdB-R	AGGTGGCGTACGTGGAGAAC	
selR-F	CGACCCGGAGCAGTACAA	*selR*
selR-R	GCAGCAGATGCAGTGGTAGA	
pvdD-F	TTGAGCGTGAGGACATTCTG	*pvdD*
pvdD-R	TCGAACCCTTGCAGGTAATC	
pvdE-F	ACGTGGGGTGATCAATGAAT	*pvdE*
pvdE-R	TTTTGCCCCACATAATTGGT	
pvdO-F	AAGAAAACCGGCCATCACTA	*pvdO*
pvdO-R	TGTCCACTCGTAGACGTTGC	
pvdS-F	ACCATCACGTCATCGTTCAA	*pvdS*
pvdS-R	TTCTCCAGCGTCGAAAAGTT	
pvdQ-F	TGCGTTTCTACGAGATGCAC	*pvdQ*
pvdQ-R	AAATAGCGAGTCGGGTCCTT	
phcf-F	ACGGCTACCAACAAATCCTG	*phcF*
phcf-R	CACCAGCAGATCCACTGAGA	
desC-F	ACTGACCGGGCTGTACGA	*desC*
desC-R	CTTCTCCGGCTTCTGGATCT	
sam0552-F	CTTCGTCCTGCAGAACTTCC	*sam0552*
sam0552-R	AGTACGCGCAGGTAGTCGTC	
foxI-F	ACGTGCGTTGATTCTGGAAT	*foxI*
foxI-R	GCACATAACAGTGATAAAGC	
foxR-F	CGGCAGGCATTGAAGGTATT	*foxR*
foxR-R	CGCTATCGGTATTGAGCTGC	
foxA-F	GCAGACAACGTGATCGAGAA	*foxA*
foxA-R	CACACCCTTCAGTCCAACCT	
pepSY-F	GTTTCTGATGATGGCGGGAC	*pepSY*
pepSY-R	ACTTGCAGTTTGGGATGCTG	
fur-F	CCAGTTTGAAGCAGCAGGAC	*fur*
fur-R	TCCACCAAATCGAATCCATGC	
EZR1[a]	ATGCGCTCCATCAAGAAGAG	
EZL2[a]	TCCAGCTCGACCAGGATG	
gfptns2[b]	ATCACCTTCACCCTCTCCAC	
nCEKG2A[c]	GGCCACGCGTCGACTAGTAC NNNNNNNNNNAGAG	
nCEKG2B[c]	GGCCACGCGTCGACTAGTAC NNNNNNNNNNACGCC	
nCEKG2C[c]	GGCCACGCGTCGACTAGTAC NNNNNNNNNNATAT	
CEKG4[d]	GGCCACGCGTCGACTAGTAC	

[a] Tn*5062*-specific primer.
[b] For first- and second-round PCR of transposon mutants.
[c] For first-round PCR of transposon mutants.
[d] For second-round PCR of transposon mutants.

nested-PCR-based sequencing approach. First-round PCR was performed using the primers gfptns2, nCEKG2A, nCEKG2B, and nCEKG2C at a ratio of 3:1:1:1. The PCR conditions were 94°C for 5 min, followed by cycles of 94°C for 30 s and 62°C for 30 s, lowered in successive cycles by 1°C until 54°C was reached, and 72°C for 3 min. A further 27 cycles of annealing at 54°C were performed, followed by a final extension of 72°C for 3 min. A second-round PCR was performed using the primers gfptns2 and CEKG4, followed by sequencing using the primer gfptnS2.

For RNA extraction, cells were collected from streaks grown on 26A agar medium for 2 days at 26.5°C. *Streptomyces* was grown on cellophane membranes. The extraction was performed with an Aurum Total RNA minikit (Bio-Rad) according to the manufacturer's instructions, except for the addition, during cell lysis, of a sonication step (3 times for 10 s each time) at high frequency using a Bioruptor apparatus (Diagenode). RNAs were eluted in a final volume of 30 μl and quantified with a NanoDrop-1000 apparatus. Before reverse transcription, RNA samples were treated with DNase I (1 U; Fermentas) in the presence of 10 U of Ribolock RNase Inhibitor (Fermentas) per 1 μg of RNA. The DNase I was then inactivated at 65°C for 10 min in the presence of EDTA (2.3 mM). The absence of residual genomic DNA was verified by a 35-cycle PCR using RNAs as templates and the primer pairs hrdB-F/hrdB-R and selR-F/selR-R for *Streptomyces* and *Pseudomonas*, respectively. Reverse transcription was performed with an iScript Advanced cDNA synthesis kit for real-time quantitative PCR (RT-qPCR) (Bio-Rad) according to the manufacturer's instructions. The sequences of the primer pairs used to amplify cDNAs and their target genes are listed in Table 2. RT-qPCRs were carried out on a CFX96 (Bio-Rad) with microplates (Multiplate 96-well unskirted PCR plates, low profile; Bio-Rad) covered with a film (Microseal Bb adhesive seals; Bio-Rad). The reaction mixture was composed of 5 μl of SYBR green Supermix (Bio-Rad), 0.2 μM each primer pair, and 4 μl of cDNA diluted 1/10. The qPCR conditions were as follow: 30 s at 95°C and 40 cycles of 5 s at 95°C and 30 s at 60°C. To verify the absence of secondary products, melting curves were produced from 65 to 95°C with an increase of 0.5°C/cycle. Total RNA levels were normalized using transcripts from the housekeeping genes *hrdB* (26) for *Streptomyces* and *selR* (MHB_002629 [27]) for *Pseudomonas* as a control. The gene *hrdB* encodes the major sigma factor of *Streptomyces* and was used as an internal control to quantify the relative expression of target genes, as it is expressed fairly constantly throughout growth (26, 28). *selR* was defined, with two other genes, as a potential housekeeping gene based on microarray data (accession number GSE38243 on the Gene Expression Omnibus at NCBI [27]) and was chosen as a reference after checking the stability of its expression in the present setup. Both *hrdB* and *selR* transcript levels were experimentally confirmed to be stable under our growth conditions (data not shown).

For each treatment, at least three biological replicates were performed, and the average and standard deviation of the expression relative to that of the reference gene *hrdB* or *selR* of each transcript were calculated (29). One-factor analyses of variance (ANOVA) were done with R to test for differences in transcript levels between treatments.

Construction of *S. coelicolor* M512 *desD* and *cchH* mutant strains. The cosmids C105.2.E01 and F76.2.F08 from the *S. coelicolor* transposon insertion single-gene knockout library (30) were used to replace the wild-type alleles of *desD* and *cchH*, respectively, in *S. coelicolor* M512. The mutated cosmids were introduced into *S. coelicolor* M512 by intergenic conjugation from *E. coli* ET12567/pUZ8002 (31). Gene replacements were confirmed by PCR analysis using the flanking and internal primers desC-F and EZR1 for the mutant *S. coelicolor* M512 *desD* and sam0552-R and EZL2 for the mutant *S. coelicolor* M512 *cchH*.

RESULTS

***P. fluorescens* BBc6R8 does not produce its siderophore pyoverdine in the vicinity of *S. ambofaciens* ATCC 23877.** *P. fluorescens* BBc6R8 produced a green-yellow pigment and fluoresced under UV light when grown on iron-depleted 26A agar medium (Fig. 1a and b). When the medium was supplemented with $FeSO_4 \cdot 7H_2O$ (12.5 μM), *P. fluorescens* BBc6R8 no longer fluoresced (Fig. 1d). This green-yellow pigmentation reflects the presence of the pyoverdine siderophore produced by *P. fluorescens* BBc6R8 under conditions of iron limitation (32). During the screening of pairwise interactions between *S. ambofaciens* ATCC 23877 and other soil bacteria, we observed that when grown on 26A agar medium in close proximity to *S. ambofaciens*, *P. fluorescens* BBc6R8 partially lost its ability to fluoresce (Fig. 1c, e, and f). Similar results were observed in the presence of the high-affinity iron chelator 2,2′-bipyridyl (200 μM) in the agar medium (see Fig. S1 in the supplemental material). Interestingly, a stronger effect was observed when *S. ambofaciens* ATCC 23877 was cultivated on 26A agar for 30 h before the petri dish was inoculated with strain BBc6R8. Under these conditions, neither the green-yellow pigment nor fluorescence could be observed (Fig. 1g). Similar results

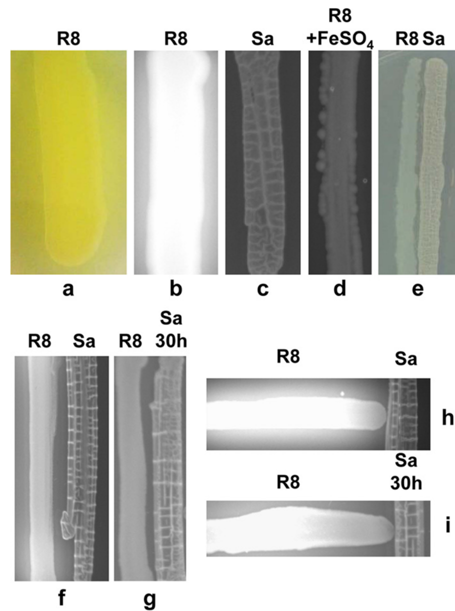

FIG 1 Effect of *S. ambofaciens* ATCC 23877 on green-yellow fluorescent pigment production by *P. fluorescens* BBc6R8. (a and b) *P. fluorescens* BBc6R8 (R8). (c) *S. ambofaciens* ATCC 23877 (Sa). (d) *P. fluorescens* BBc6R8 cultivated on medium supplemented with $FeSO_4$. (e and f) *P. fluorescens* BBc6R8 plus *S. ambofaciens* ATCC 23877. (g) *P. fluorescens* BBc6R8 plus *S. ambofaciens* ATCC 23877 that had been streaked on a plate 30 h before strain BBc6R8. (h) *P. fluorescens* BBc6R8 plus *S. ambofaciens* ATCC 23877 cocultivated perpendicularly. (i) *P. fluorescens* BBc6R8 plus *S. ambofaciens* ATCC 23877 (streaked on a plate 30 h before strain BBc6R8) cocultivated perpendicularly. (b, c, d, f, g, h, and i) The plates were visualized under UV light. The images were taken from below the plates after a 2-day (co)culture with incubation at 26.5°C on 26A agar medium.

were obtained when 2,2′-bipyridyl was present in the agar medium (see Fig. S1 in the supplemental material). The negative effect on pyoverdine production was proportional to the lag between the inoculations of the two bacteria on the plate. In addition, streaking the bacterial partners perpendicular to one another revealed that the effect of *S. ambofaciens* on pyoverdine production by *P. fluorescens* occurred only in the area surrounding the streak of *Streptomyces* (Fig. 1h and i). Altogether, these data show that in the presence of *S. ambofaciens* ATCC 23877, strain BBc6R8 does not produce its own siderophore.

Transcription of the pyoverdine and enantiopyochelin synthesis genes is no longer induced in the presence of *S. ambofaciens* ATCC 23877. The biosynthesis and uptake of pyoverdine, as well as their regulation, have been thoroughly studied in *P. aeruginosa* (33–36) and in *P. fluorescens* SBW25 (10). A search in the *P. fluorescens* BBc6R8 genome sequence (27) indicated that all the genes involved in these processes are conserved in the strain (see Table S1 in the supplemental material), suggesting similar mechanisms of biosynthesis, uptake, and regulation in strain BBc6R8 and in *P. aeruginosa*. Therefore, we tested the influence of *S. ambofaciens* on the expression of these genes by varying the duration of preincubation of *Streptomyces* on the plate before the addition of *Pseudomonas*. Messenger RNAs from cell lysates of *P. fluorescens* BBc6R8 grown on 26A agar medium in the absence or presence of *S. ambofaciens* were quantified using RT-qPCR. The duration of preincubation of *Streptomyces* varied between 0 and 54 h before strain BBc6R8 was streaked on the plate. The expression of genes involved in the biosynthesis of pyoverdine (*pvdD*) or its regulation (*pvdO*, *pvdS*, and *pvdQ*) and export (*pvdE*) and in the biosynthesis of the second type of siderophore of *P. fluorescens* BBc6R8, enantiopyochelin (*phcF*), were monitored after 2 days of growth. As expected, all the siderophore-related genes were transcribed when *Pseudomonas* was cultivated alone, reflecting the iron limitation in the 26A medium. In contrast, transcript levels of the pyoverdine genes were significantly reduced when *P. fluorescens* BBc6R8 was grown in the presence of *S. ambofaciens* (Fig. 2) ($P < 0.01$; one-factor ANOVA), except for *pvdD* and *pvdS*, when the two bacterial strains were simultaneously streaked on the agar plate. As for the production of pyoverdine, the decrease in transcript levels was proportional to the time lag between the seeding of the two bacterial strains on the plate for all genes analyzed. The expression of the enantiopyochelin gene *phcF* was similarly affected by the presence of *S. ambofaciens* ATCC 23877. Altogether, these data confirm our initial observation that *P. fluorescens* BBc6R8 reduces and even stops the production of its own siderophores on iron-limited medium in the presence of *S. ambofaciens* ATCC 23877.

***S. ambofaciens* ATCC 23877 expresses genes necessary for desferrioxamine and coelichelin production on 26A medium.** *S. ambofaciens* ATCC 23877 produces two types of tris-hydroxamate siderophores: desferrioxamine (B and E) and coelichelin (37). We hypothesized that *S. ambofaciens* produces these siderophores when grown on 26A agar medium and that the siderophores would then be accessible to strain BBc6R8. To test this hypothesis, we analyzed the expression of the genes of the biosynthesis pathways of the two siderophores by RT-qPCR. The transcription of *desC* (acyl-coenzyme A [CoA] acyltransferase; desferrioxamine) (37) and *samR0552-cchH* (nonribosomal peptide synthetase [NRPS]; coelichelin) (37) was monitored over a 2.5-day time course. Both genes were expressed under these conditions, and their transcription peaked at around 30 h (Fig. 3). This indicates that *S. ambofaciens* also detects iron deficiency on 26A medium and induces the expression of its siderophore-biosynthetic genes, strongly suggesting that desferrioxamine and coelichelin are produced.

***P. fluorescens* BBc6R8 utilizes siderophores produced by *Streptomyces*.** Consequently, *P. fluorescens* BBc6R8 could use ferrioxamine(s) and/or ferricoelichelin as a xenosiderophore to cope with the lack of iron in the 26A medium. To test this hypothesis, *P. fluorescens* BBc6R8 was cultivated on a 26A agar plate supplemented with desferrioxamine B mesylate at 200 μM. After 48 h of culture, *P. fluorescens* BBc6R8 did not fluoresce in the presence of the exogenous purified siderophore, in contrast to the control (Fig. 4a and b). The addition of 8 μM desferrioxamine B to the medium was sufficient to inhibit pyoverdine production (Fig. 4c). This indicates that *P. fluorescens* BBc6R8 stops producing its own siderophore in the presence of desferrioxamine B, likely utilizing the compound as a xenosiderophore.

Streptomyces coelicolor A3(2) produces the same siderophores as *S. ambofaciens* ATCC 23877 (37–39). Since cosmids with the biosynthetic *desD* (type C siderophore synthetase) (37) and *cchH* (NRPS) (39) genes mutated are available from the *S. coelicolor* transposon insertion single-gene knockout library (30), we decided to disrupt these genes in *S. coelicolor* M512 (a derivative of the *S. coelicolor* A3(2) M145 strain unable to produce the pigmented antibiotics actinorhodin and undecylprodigiosine [40]). Pairwise cultures of *P. fluorescens* BBc6R8 and *S. coelicolor cchH*

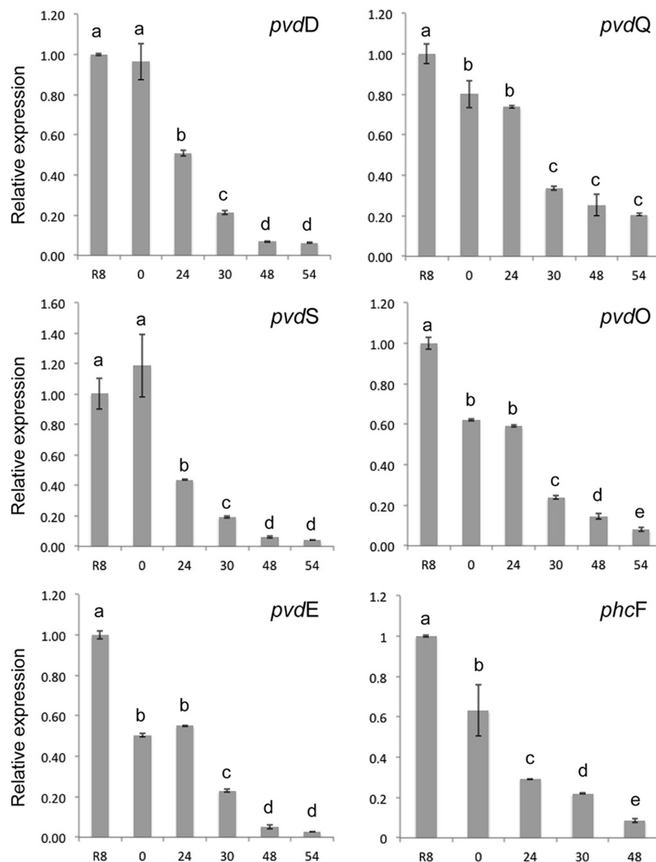

FIG 2 Effect of *S. ambofaciens* ATCC 23877 on the expression levels of *P. fluorescens* BBc6R8 genes involved in the biosynthesis of pyoverdine and enantiopyochelin as measured by RT-qPCR. The expression levels are expressed as the transcript levels of the target genes relative to the transcript level of the housekeeping gene *selR*, which is stable under the tested growth conditions. The data are expressed as the mean value of three biological replicates. The error bars denote standard errors. For each transcript, values with the same letter are not significantly different according to a one-factor ANOVA ($P > 0.01$). R8, *P. fluorescens* BBc6R8 grown on 26A agar medium in the absence of *S. ambofaciens*; 0 to 54, *P. fluorescens* BBc6R8 grown in the presence of *S. ambofaciens* ATCC 23877 with *S. ambofaciens* streaked on the 26A plate 0 h, 24 h, 30 h, 48 h, or 54 h before strain BBc6R8. Total RNAs were extracted 48 h after the plates were inoculated with *P. fluorescens* and incubated at 26.5°C.

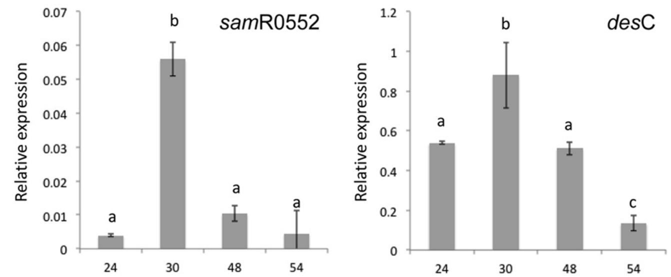

FIG 3 Expression levels of *S. ambofaciens* ATCC 23877 genes controlling the synthesis of desferrioxamine B and E and coelichelin as measured by RT-qPCR. The *desC* gene (right) is involved in the synthesis of desferrioxamines, and the *samR0552* gene (left) is involved in coelichelin biosynthesis. Total RNAs were extracted after 24, 30, 48, and 54 h of growth on 26A agar medium at 26.5°C. The expression levels are shown as the transcript levels of the target genes relative to the transcript level of the housekeeping gene *hrdB*, which is stable under the tested growth conditions. The data are expressed as the mean value of three biological replicates. The error bars denote standard errors. For each transcript, values with the same letter are not significantly different according to a one-factor ANOVA ($P > 0.01$).

and *desD* mutants revealed that *Pseudomonas* did not fluoresce in the presence of either of the two single mutants after 2 days of culture (Fig. 4d and e). However, in the presence of the *S. coelicolor* Δ*des* Δ*cch* strain, a double mutant deficient in the biosynthesis of desferrioxamine and coelichelin (37), the production of the green-yellow pigment by *P. fluorescens* was not affected (Fig. 4f). Therefore, our data suggest that *P. fluorescens* BBc6R8 has the ability to recognize and to take up both ferrioxamine and ferricoelichelin and consequently no longer produces its own siderophores, pyoverdine and enantiopyochelin.

***Streptomyces* is unable to take up *P. fluorescens* BBc6R8 siderophores.** The question of whether *Streptomyces*, conversely to *Pseudomonas*, is able to use pyoverdine and/or enantiopyochelin as a xenosiderophore arose. It has been reported that the *S. coelicolor* Δ*des* Δ*cch* strain (strain W13) cannot grow on agar medium supplemented with 200 μM 2,2′-bipyridyl due to an extreme iron deficiency (37). Indeed, in our experimental setup, it was unable to grow on a 26A plate containing the iron chelator (data not shown). Therefore, we examined whether *P. fluorescens* BBc6R8 could compensate for this deficiency by providing its own siderophores. A plug was collected from a 3-day culture of BBc6R8 on a 26A agar plate with or without 2,2′-bipyridyl (200 μM) and placed onto a 26A agar plate supplemented with the iron chelator (200 μM) with the W13 strain evenly spread on it. Alternatively, strain W13 was streaked on a 26A plate containing 2,2′-bipyridyl (200 μM) alongside the BBc6R8 strain, inoculated on the plate 3 days earlier. As a control, similar experiments were carried out with *S. coelicolor* M512 instead of *P. fluorescens* BBc6R8. While M512 efficiently promoted the growth of W13, BBc6R8 had no effect (see Fig. S2 in the supplemental material). Therefore, we conclude that *S. coelicolor* is unable to use pyoverdine and enantiopyochelin as xenosiderophores. By extension, we expect similar behavior for *S. ambofaciens*, since the two species encode the same extracellular siderophore binding proteins (41 and data not shown).

***P. fluorescens* BBc6R8 uses a TonB-dependent receptor to detect *S. ambofaciens* ATCC 23877 ferrisiderophores.** To identify the genes of *P. fluorescens* BBc6R8 involved in the uptake of ferrioxamines and ferricoelichelin, pairwise cultures of *S. ambofaciens* ATCC 23877 and 4,400 clones from a transposon mutant library of *P. fluorescens* BBc6R8 (P. Burlinson and A. Deveau, personal communication) were performed on solid 26A medium. *S. ambofaciens* was streaked on the plates 30 h before the *Pseudomonas* mutants to allow accumulation of ferrioxamines and ferricoelichelin in the medium. Two mutants, P28H6 and P18B10, still fluoresced under UV light under these conditions (Fig. 5a to d). Similar results were observed when the P28H6 and P18B10 clones were grown near the *S. coelicolor* M512 *desD* and *cchH* mutants that were unable to produce desferrioxamine and coelichelin, respectively (Fig. 5e and f). In addition, production of pyoverdine was also observed in the two *Pseudomonas* mutants when cultivated on 26A agar plates containing 200 μM desferrioxamine B (Fig. 5g and h). Therefore, we concluded that the mutants P28H6 and P18B10 of *P. fluorescens* BBc6R8 are no longer able to recognize and/or to take up the *Streptomyces* siderophores. Analysis of the mutants revealed that both P28H6 and P18B10 have the same

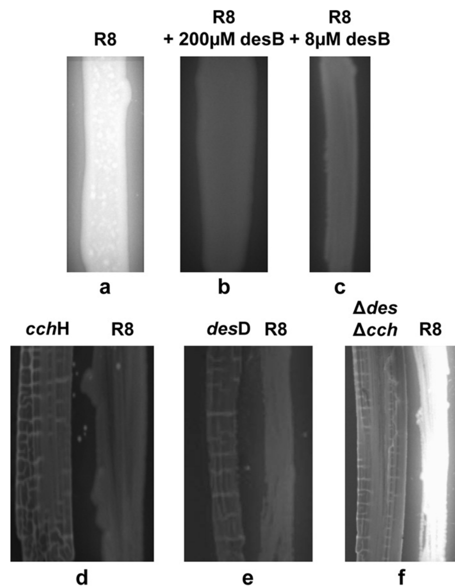

FIG 4 Effects of desferrioxamine and coelichelin on the production of pyoverdine by *P. fluorescens* BBc6R8. (a) *P. fluorescens* BBc6R8 (R8). (b) *P. fluorescens* BBc6R8 cultivated in the presence of 200 μM desferrioxamine B mesylate. (c) *P. fluorescens* BBc6R8 cultivated in the presence of 8 μM desferrioxamine B mesylate. (d) *P. fluorescens* BBc6R8 plus *S. coelicolor* M512 *cchH*. (e) *P. fluorescens* BBc6R8 plus *S. coelicolor* M512 *desD*. (f) *P. fluorescens* BBc6R8 plus *S. coelicolor* Δ*des* Δ*cch*. The *Streptomyces* strains were streaked on plates 30 h before the inoculation of *P. fluorescens*. The images were taken under UV light from below the plates after a 2-day (co)culture on 26A agar medium at 26.5°C.

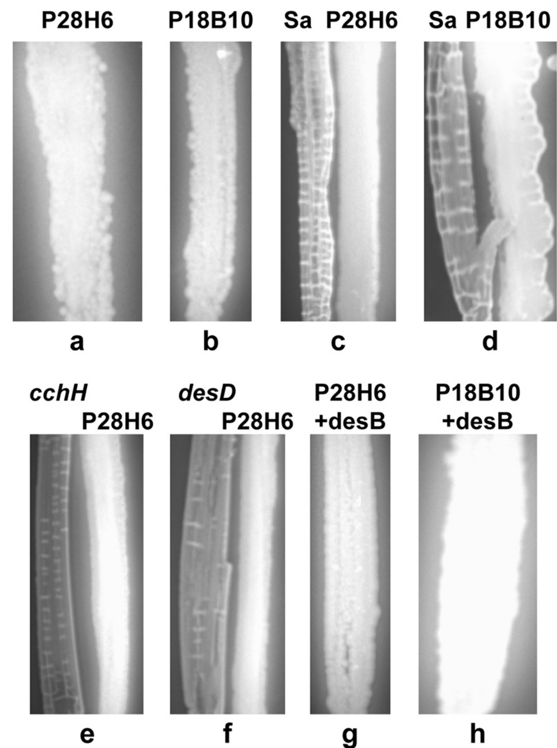

FIG 5 Effect of the disruption of the *foxA* gene on pyoverdine production by *P. fluorescens* BBc6R8. (a) *P. fluorescens* P28H6. (b) *P. fluorescens* P18B10. (c and d) *P. fluorescens* P28H6 (c) and *P. fluorescens* P18B10 (d) cultivated with *S. ambofaciens* ATCC 23877 (Sa). (e and f) *P. fluorescens* P28H6 cultivated with *S. coelicolor* M512 *cchH* (e) and with *S. coelicolor* M512 *desD* (f). (g and h) *P. fluorescens* P28H6 (g) and *P. fluorescens* P18B10 (h) cultivated in the presence of 200 μM desferrioxamine B mesylate. The *Streptomyces* strains were streaked on plates 30 h before the inoculation of *P. fluorescens*. The images were taken under UV light from below the plates after a 2-day (co)culture at 26.5°C on 26A agar medium.

gene mutated, the MHB_05767 gene (see Fig. S3 in the supplemental material). A BLASTp search showed that the product of this gene shares 88%, 84%, and 67% identity with FoxA, a TonB-dependent ferrioxamine B receptor, of *P. fluorescens* Pf0-1, *Pseudomonas protegens* Pf-5, and *P. aeruginosa* PAO1 (42, 43), respectively (see Fig. S4 in the supplemental material). By analogy, we named the gene of *P. fluorescens* BBc6R8 *foxA*. From this set of experiments, we conclude that *P. fluorescens* BBc6R8 would be able to detect and to take up the siderophores ferrioxamine B and ferricoelichelin via the outer membrane receptor FoxA.

***S. ambofaciens* ATCC 23877 induces expression of genes involved in TonB-dependent receptor synthesis.** In *P. fluorescens* Pf0-1, *P. protegens* Pf-5, and *P. aeruginosa* PAO1, *foxA* belongs to an operon with the genes *foxI* (encoding an ECF sigma factor), *foxR* (encoding an anti-sigma factor), and *pepSY* (encoding a PepSY TM helix protein), which is under the regulation of the Fur protein (43). Analysis of the genome sequence of *P. fluorescens* BBc6R8 indicated that the homologs of the genes *foxI*, *foxR*, and *pepSY* were also present in BBc6R8 (see Table S1 in the supplemental material) and were organized in a cluster similar to those in *P. fluorescens* Pf0-1, *P. protegens* Pf-5, and *P. aeruginosa* PAO1. A homolog of the *fur* gene was also retrieved in the genome sequence of the BBc6R8 strain (see Table S1 in the supplemental material). This suggests that the mechanism of regulation of the desferrioxamine-mediated iron uptake system in *P. fluorescens* BBc6R8 is similar to that of *P. aeruginosa* (33, 35). After 24 h of incubation with *S. ambofaciens*, the expression levels of the *foxA*, *foxI*, *foxR*, and *pepSY* genes increased compared to the control treatment ($P < 0.01$; one-factor [R8 alone] ANOVA) (Fig. 6). The transcription level of *fur* was significantly modified by the presence of *S. ambofaciens*, although the fold change was much smaller than for the other transcripts (Fig. 6) ($P < 0.01$; one-factor [R8 alone] ANOVA). These data show that in the presence of *S. ambofaciens*, and under conditions stimulating siderophore production, the *P. fluorescens* BBc6R8 *foxA* transcriptional-regulation cascade is induced, most likely resulting in the production of the FoxA receptor and in the uptake of the *Streptomyces* siderophores.

Utilization of desferrioxamine is shared among *P. aeruginosa* and other fluorescent pseudomonads. As similar biosynthetic and uptake systems are present in *P. fluorescens* BBc6R8, *P. aeruginosa* PAO1 (9, 42), *P. protegens* Pf-5, and *P. fluorescens* Pf0-1 (43), we expected that these bacterial strains might also be able to react to the presence of the siderophores produced by *S. ambofaciens* ATCC 23877. Indeed, we found that *P. fluorescens* Pf0-1, *P. aeruginosa* PAO1, and *P. protegens* Pf-5 no longer produced a green pigment in the presence of *S. ambofaciens* ATCC 23877 and did not fluoresce under UV light. Interestingly, *P. fluorescens* SBW25, which does not have the *foxA* gene, fluoresced even in the presence of *S. ambofaciens* (see Fig. S5 in the supplemental material). In the presence of purified desferrioxamine B, *P. fluorescens* Pf0-1, *P. aeruginosa* PAO1, and *P. protegens* Pf-5 no longer fluoresced, again in contrast to strain SBW25 (see Fig. S5 in the supplemental material). In cocultures with *S. coelicolor* M512 *desD*, *P. fluorescens* Pf0-1, *P. aeruginosa* PAO1, and *P. protegens* Pf-5 fluo-

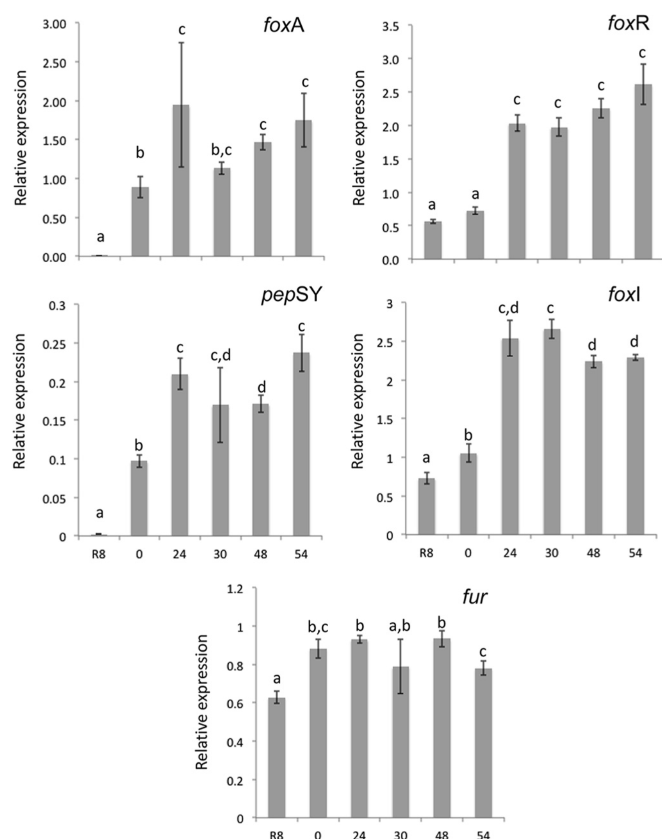

FIG 6 Effects of *S. ambofaciens* ATCC 23877 on the expression levels of *P. fluorescens* BBc6R8 genes controlling the synthesis of the FoxA receptor. Analysis was done by RT-qPCR. R8, *P. fluorescens* BBc6R8 grown on 26A agar in the absence of *S. ambofaciens*; 0 to 54, *P. fluorescens* BBc6R8 grown in the presence of *S. ambofaciens* ATCC 23877 that was streaked on solid 26A plates 0 h, 24 h, 30 h, 48 h, or 54 h before strain BBc6R8. The expression levels are expressed as the transcript levels of the target genes relative to the transcript level of the housekeeping gene *selR*, which is stable under the tested growth conditions. The data are expressed as the mean value of three biological replicates. The error bars denote standard errors. For each transcript, values with the same letter are not significantly different according to a one-factor ANOVA ($P > 0.01$).

resced less than in single culture, suggesting that they are also able to utilize coelichelin (see Fig. S5 in the supplemental material). Interestingly, a screening of a library of 60 fluorescent pseudomonad strains isolated from forest soil (Table 1) revealed that all the strains were also able to utilize *S. ambofaciens* siderophores (data not shown).

DISCUSSION

Our study reports interspecies adaptive behavior during common utilization of limited iron resources between the two soil inhabitants *Pseudomonas* and *Streptomyces*. In particular, we show that, under iron-deficient conditions, *P. fluorescens* BBc6R8 does not induce the production of the fluorescent siderophore pyoverdine in the presence of *S. ambofaciens* ATCC 23877. Instead, *P. fluorescens* very likely utilizes the *S. ambofaciens* siderophores desferrioxamine B and coelichelin as xenosiderophores, thanks to its FoxA receptor. In contrast to most studies, in which the use of xenosiderophores was revealed indirectly through the addition of purified siderophore to the growth medium (44–47), binding affinity assays (11, 48), or native PAGE and surface plasmon resonance (49) or with labeled iron (11, 50, 51), we revealed potential iron piracy through a direct interaction between a Gram-positive and a Gram-negative bacterium. This potential piracy likely occurs only in one direction. Indeed, the growth of an *S. coelicolor* Δ*des* Δ*cch* mutant under iron-deficient conditions could not be rescued in the presence of *P. fluorescens* BBc6R8 (see Fig. S2 in the supplemental material). Similar behavior is expected from *S. ambofaciens* ATCC 23877, since the strain is extremely close phylogenetically to *S. coelicolor* and they encode the same extracellular siderophore binding proteins. Similar piracy involving streptomycetes has been reported previously, but only with other actinomycetes (e.g., *Streptomyces* and *Amycolatopsis*) (15, 44, 52). It is interesting that these examples also involved desferrioxamines and that competition for iron could either stimulate (44, 52) or curtail (15) the growth and/or development of *Streptomyces*. The effect of the siderophore piracy by *P. fluorescens* on *S. ambofaciens* remains to be identified. Indeed, we could not observe any effect on the morphological differentiation of *S. ambofaciens*, as it forms only vegetative mycelium on the 26A medium.

Although our model bacteria were isolated from independent ecological niches (*Laccaria bicolor* sporocarp for *P. fluorescens* BBc6R8 [53] and soil in the Picardie region, France, for *S. ambofaciens* [18]) and the experiments were carried out under laboratory growth conditions, this interaction for iron capture between *Streptomyces* and *Pseudomonas* likely occurs in natural environments. Indeed, several studies have reported that these bacterial genera share common ecological niches within soils, including environments such as the rhizosphere or bulk soils (54–56). Therefore, they are expected to use common pools of scarce but essential elements, such as iron. The piracy would occur through the production by *P. fluorescens* BBc6R8 of the TonB-dependent receptor FoxA. Interestingly, strain BBc6R8 does not produce ferrioxamine, which is the most effective compound for iron scavenging, followed by pyoverdine and enantiopyochelin, both of which it does produce (56). In a competitive environment, such as soil, it is certainly more advantageous to use efficient chelators produced by its neighbors rather than to produce its own siderophores, especially if they are less effective and metabolically costly, as demonstrated previously (6). In addition, since desferrioxamines are synthesized by many soil organisms, in this way, *P. fluorescens* BBc6R8 could obtain iron at a low energy cost in many competitive situations. Interestingly, it should be noted that purified desferrioxamine added to the culture medium had a positive effect on the growth of *P. fluorecens* BBc6R8 (data not shown). In contrast, *S. ambofaciens* negatively impacted the growth of BBc6R8 in cocultures, presumably because of the secretion of secondary metabolites and/or through nutrient competition (data not shown).

The production of desferrioxamines seems to be conserved throughout streptomycetes (57, 58). The *desABCD* genes that direct the synthesis of these chelating agents (38, 59) have been found in all *Streptomyces* sequenced genomes and in other, related genera, such as *Salinispora* (60). Furthermore, Kobayakawa and Kodani detected by high-performance liquid chromatography (HPLC) the production of desferrioxamines in 78% of their *Streptomyces* collection (61). Desferrioxamines are also produced by other actinomycetes and some Gram-negative bacteria (62, 63).

Our data indicate that the BBc6R8 outer membrane receptor FoxA would allow the uptake not only of desferrrioxamines of *S.*

coelicolor, but also of the hydroxamate siderophore ferricoelichelin. The ability seems to be widespread among fluorescent pseudomonads. Indeed, the FoxA receptor is present in three different subclades of the genus *Pseudomonas* (64), and based on our screening of 60 *P. fluorescens* environmental strains, it is likely that they also possess a *foxA* gene and even a *foxA* operon. A BLASTp analysis also showed that FoxA homologs are present in other *Pseudomonas* species, such as *P. stutzeri*, *P. aeruginosa*, *Pseudomonas fulva*, *Pseudomonas putida*, and *Pseudomonas resinovorans* (data not shown), and also in members of different genera of bacteria, like *Yersinia enterocolitica* (12) and *Erwinia herbicola* (13). In these genera, FoxA is known to bind a collection of ferrioxamine derivatives with different chain lengths or bridges (65). The tris-hydroxamate siderophore coprogen is also recognized to a certain extent by FoxA (65). Desferrioxamine B, coelichelin, and coprogen are linear siderophores (37, 65). These data suggest that FoxA could bind other linear ferric-tris-hydroxamate siderophores (65). Therefore, *P. fluorescens* BBc6R8 may have the ability to obtain a wider variety of siderophores than were investigated in this study through the production of the FoxA receptor and thus to very efficiently compete with other bacteria. FoxA could then be considered an outer membrane receptor with a broad spectrum, at least for siderophores of the tris-hydroxamate family, and as a widespread receptor. Other bacteria, such as *Streptomyces*, also possess broad-spectrum tris-hydroxamate receptors. Indeed, in *S. ambofaciens* ATCC 23877 and *S. coelicolor* A3(2), CdtB, a siderophore binding protein involved in iron-siderophore transport, is able to bind ferrioxamines and ferricoelichelin with high affinity (37, 41). Moreover, DesE, a second siderophore binding protein, binds different ferric-tris-hydroxamates, with the exception of ferricoelichelin (41). Possessing some broad-spectrum siderophore receptors may be a frequent strategy among microorganisms selected during evolution to be more competitive in soil.

The capability to take up xenosiderophores can be amplified if bacteria possess multiple xenosiderophore receptors. This property is particularly well developed in the genus *Pseudomonas*. Indeed, several *Pseudomonas* species possess numerous TonB-dependent receptors, usually more than 20, with *P. protegens* Pf-5 encoding the highest number (45) of TonB-dependent receptors (9). Twenty-nine have been identified so far in the draft genome sequence of the BBc6R8 strain (27). Several bacteria possessing multiple siderophore binding proteins, like *P. fragi*, have even lost the ability to produce siderophores and rely exclusively on siderophore piracy (11). Whether siderophore piracy results from the loss of the ability to synthesize the cognate siderophores during evolution or the acquisition of receptor genes through horizontal transfer remains an open question. Nevertheless, our data and those of others strongly support the idea that the capacity for siderophore piracy is widespread among bacteria and that piracy by "cheaters" does happen *in vivo* (66). What is unclear is to what extent this piracy really occurs in natural ecosystems such as soils and how it impacts community dynamics. Metagenomics combined with metatranscriptomics may help to answer this question in the future by providing a more complete picture of who is producing what and who is "cheating" in natural complex microbial communities.

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