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Desferrioxamine-dependent iron transport in *Erwinia amylovora* CFBP1430: cloning of the gene encoding the ferrioxamine receptor FoxR

Rémy Kachadourian, Alia Dellagi*, Jacqueline Laurent*, Laurent Bricard, Gerhard Kunesch & Dominique Expert*

*Laboratoire de Chimie Bioorganique et Bioinorganique, CNRS URA 1384, Université Paris-Sud, Orsay and *Laboratoire de Pathologie Végétale, INRA/INA P-G, Paris, France*

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Iron deprivation of *Erwinia amylovora* CFBP1430, a species causing fire blight on Pomoïdeae, was shown to induce the production of siderophores of the desferrioxamine (dfo) family and two outer membrane polypeptides with apparent molecular weight of about 70 and 80 kDa, respectively. Cyclic dfo E was characterized as the major metabolite. Phage Mu*dII*pR13 insertional mutagenesis and screening on CAS-agar medium yielded three dfo non-producing and one overproducing clones. These clones failed to grow in the presence of the Fe(III) chelator EDDHA and were determined further as dfo and ferrioxamine transport negative mutants, respectively. The transport mutant which appeared to lack the 70 kDa polypeptide in the outer membrane allowed the purification of dfo E. Growth under iron limitation of dfo negative mutants was stimulated with ferrioxamine E and B but not with other ferrisiderophores tested. The host DNA sequence flanking the left terminal part of the Mu*dII*pR13 prophage responsible for the transport mutation was cloned and used to probe a parental gene library by DNA–DNA hybridization. Two recombinant cosmids restoring the transport mutation to normal were identified. Both cosmids also conferred the ability to utilize ferrioxamine B and E as iron sources on a FhuE$^-$ mutant of *Escherichia coli*. This correlated with the production of an additional polypeptide of 70 kDa in the outer membrane of *E. coli* transconjugants, thus confirming that this protein serves the ferrioxamine receptor function (FoxR) in *E. amylovora*.

Keywords: desferrioxamines, *Erwinia amylovora*, ferrioxamine receptor, iron transport, pathogenicity.

Introduction

The role of iron in vertebrate host infections has been studied extensively (Bullen & Griffith 1987). In microorganisms, excretion of siderophores and the specific transport of their ferric complexes is commonly used to overcome the conditions of low iron availability during infection. The iron complexing proteins responsible for the transport of the metal within body fluids may prevent proliferation of a pathogen through a bacteriostatic effect, i.e. by depriving it of nutritional iron (Weinberg 1984). One practical aspect emerging from the numerous studies related to iron transport is the development of a new class of bacterial drugs based on the concept of delivering, with high affinity and specificity, siderophore–antibiotic conjugates through bacterial iron transport systems (Bullen & Griffith 1987, Miller 1989). The natural antibiotic albomycin which is taken up by *Escherichia coli* via the ferrichrome transport system (Hartmann *et al.* 1979) provides a rationale for this concept.

While siderophores are well-established virulence factors in vertebrate diseases, competition for iron is less well documented in plant pathogenesis. The case of the soft rot caused by *Erwinia chrysanthemi* strain 3937 on saintpaulia plants proved to be informative. The systemic spread of symptoms in the host depends on the integrity of a high-affinity iron assimilation system (Enard *et al.* 1988) that involves the siderophore chrysobactin (Persmark *et al.* 1989). Leaf intercellular fluids are sensed as a low iron environment to which the pathogen responds by excreting chrysobactin (Neema *et al.* 1993, Masclaux & Expert 1995). Furthermore, the production of several pectinases responsible for cell wall degradation in colonized tissues is stimulated in iron-deprived *E. chrysanthemi* cells (Sauvage & Expert 1994). In contrast, the siderophore of *Ustilago maydis*, i.e. ferrichrome,

R. Kachadourian and A. Dellagi have contributed equally to this work. Address for correspondence: D. Expert, Pathologie Végétale, INRA/INAP–G, 16 rue Claude Bernard, 75231 Paris cedex 05, France. Tel: (1) 44081706; Fax: (1) 4408 1631.

does not appear to be a virulence factor because disruption of the corresponding biosynthetic pathway does not affect the pathogenicity of the fungus (Mei *et al.* 1993).

Fire blight, a slowly progressing necrosis of Pomoïdeae caused by *E. amylovora*, provides another interesting siderophore-related object. Extracellular development of the pathogen and absence of rapid host cell death (Brisset & Paulin 1992) constitute a situation in which competition for iron is likely to be important. Several strains of *E. amylovora* were previously found to produce hydroxamate siderophores under iron deficiency (Vanneste & Expert 1990). A recent analysis of a larger number of strains established that these siderophores belong to the desferrioxamines family (Feistner *et al.* 1993).

In our work, we have analysed the iron assimilation of *E. amylovora* CFBP1430 with the goals of further exploring the roles of siderophores in plant pathogenesis and the feasibility to exploit microbial iron transport systems of the control of bacterial diseases. In particular, we describe several siderophore and one transport deficient mutants, the latter having allowed the purification of ferrioxamine E, i.e. the major component of the diverse cyclic desferrioxamines characterized in strain CFBP1430. We report the cloning of a region of the *E. amylovora* chromosome encoding the receptor function for ferrioxamines which is accomplished by a protein with an apparent molecular weight of 70 kDa.

Materials and methods

Bacterial strains and growth conditions

Desferrioxamine-deficient (VD31, VD57, VD61, VD36 and VD41) and ferrioxamine transport (VD17) mutant strains described in the text are derivatives from CFBP1430, a natural isolate of *E. amylovora* (Paulin & Samson 1973). *E. coli* MS172, *araD*, *lacU*, *rpsL*, *relA*, *thi*, *fibB*, *deoC*, *ptsF*, *rbsR*, *aroB*, *fhuE*:: lplacMu53 (Sauer *et al.* 1987) was received from K. Hantke. L broth and L agar (Miller 1972) were used as rich media, MM9 (Schwyn & Neilands 1987) and Tris (Franza *et al.* 1991) media supplemented with 0.2% glucose and 0.02% nicotinic acid were used for low-iron liquid cultures. Severe iron starvation was achieved by the addition of EDDHA [ethylene dimaine-*N*, *N'*-bis(2-hydroxyphenyl-acetic acid)] deferrated by the procedure of Rogers (1973) to Tris or L medium at final concentrations of 100 and 200 $\mu g\,ml^{-1}$, respectively. Addition of ferric chloride and phosphate (1 M KH_2PO4 adjusted to pH 7 with KOH) to Tris medium at 10 μM and 100 mM final concentration, respectively, provided iron-rich conditions. Chloramphenicol was added at 20 $\mu g\,ml^{-1}$. Cultures were kept at 28°C.

Siderophore detection

Chemical assays for detecting catechol (Arnow 1937) and hydroxamate (Csàky 1948) were performed. Siderophore activity was identified as Chrome Azurol S (CAS)-reacting material in culture, supernatant fluids (Schwyn & Neilands 1987) using dfo B (Desferal; Ciba Geigy, Co.) as standard.

Isolation and phenotypic analysis of mutants

Mutagenesis of *E. amylovora* CFBP1430 was carried out with the Mu*dI*IpR13 derivative [Δ(Mu*AB*), Cm^R, *lac'ZYA*] (Ratet *et al.* 1988) as described previously (Barny *et al.* 1990). Chloramphenicol-resistant clones were replicated onto CAS-agar medium. Mutants displaying an altered phenotype on this medium as described in the text, were streaked on EDDHA-L agar medium.

Cross-feeding assay

Production and utilization of ferrioxamine was bioassayed as described previously (Enard *et al.* 1988). Utilization of ferric citrate by biosynthetic mutants as an iron source (Hussein *et al.* 1981) was tested by spotting 10 μl of various ferric citrate complex solutions (1 mM, 10 mM, 100 mM $FeCl_3$:1 M citrate final ratio) on sterile paper disks placed on EDDHA-L agar medium seeded with the strain to be tested at a final concentration of 10^5 c.f.u. ml^{-1}. The assay is positive when a halo of growth surrounding the disk can be detected even with the solution corresponding to the lowest iron:citrate ratio. Ferrichrome and ferric complexes of acrobactin, enterobactin, chrysobactin and rhodotulic acid were assayed similarly.

Outer membrane preparation analysis

Triton-insoluble walls (outer membranes) were prepared and analysed by SDS–PAGE according to Expert & Toussaint (1985).

DNA methods

The CFBP1430 genomic library constructed in vector pLA2917 and DNA techniques were described by Barny *et al.* (1990).

Extraction and purification of siderophores

Strains were grown in Tris medium. Batches of 500 ml of culture medium inoculated with 5 or 50 ml of precultures in L broth were incubated in 2 l conical flasks for 24 h, the cells were then removed by centrifugation and the hydroxamate content of supernatant fluids was determined. Batches of 500 ml were supplemented with 25 mg of ferrous sulphate and aerated for 15 h to allow ferric complex formation. Purification was carried out according to the method described by Berner *et al.* (1988) with modifications. Fluids were passed through an Amberlite XAD-2 column (500 g). After washing with two volumes of water, the siderophore ferric complexes were desorbed with 1 volume of methanol. Methanol and traces of water were removed by evaporation *in vacuo* and further drying over P_2O_5. The extracts corresponding to 1 l of bacterial culture were combined and purified by gel filtration on Sephadex LH-20 (40 g) with methanol as eluent. This procedure led to efficient elimination of nicotinic acid and preculture medium components. The main ferrioxamines were separated by

medium pressure chromatography on a silica gel column using dichloromethane:methanol:water (70:24:4) as eluent. The purity of ferrioxamine E and the presence of ferrioxamines D_2, X_1 (or X_7) and G_1 was checked by HPLC as described previously (Reissbrodt *et al.* 1990), using a C_{18} reversed-phase column (Ultrasphere-ODS, 5 μm, 250 × 4.6 mm; Altex company), an isocratic mobile phase of acetonitrile (12%) and 10 mM acetic acid/ammonium acetate buffer (pH 4) and a flow rate of 1 ml min^{-1}. The eluate was monitored at wavelengths of 220 and 365 nm using a Perkin-Elmer Diode Array detector. Ferrioxamine E was deferrated using 8-hydroxyquinoline (Wiebe & Winkelmann 1975).

Mass spectrometry

Ferrioxamine E as well as small amounts of ferrioxamines D_2, an additional cyclic ferrioxamine (m/z 625 corresponding to X_1 or X_7) and ferrioxamine G_1 were detected by a single quadrupole mass spectrometer (Nermag Model R10-10C) equipped with an electrospray source (Analytica, Branford, CT) and an API data system (Quad Service, Argenteuil, France). The solvent was acetonitrile + acetic acid 0.1%.

^1H- and ^{13}C-NMR spectroscopy

^1H- and ^{13}C-NMR spectra (D_2O) of desferrioxamine E ($c = 0.02$ M) were performed on a Bruker AM 250 spectrometer. ^1H resonances were assigned by irradiation experiments using HDO ($\delta = 4.6$ p.p.m.) as an internal standard. ^{13}C resonances were assigned by Huber's chemical shift correlations (Huber 1984) using dioxane as the internal standard ($\delta = 67.4$ p.p.m.).

Results

Identification of a hydroxamate-dependent iron uptake system in E. amylovora

When grown under conditions leading to iron starvation, strain CFBP1430 produced extracellular hydroxamate but no detectable catechol. In Tris medium, increased production of hydroxamates correlated with a CAS reacting activity reaching up to 44 μM Desferal equivalents during the stationary phase of growth. SDS–PAGE analysis of the outer membrane proteins revealed the presence of two iron repressible polypeptides of about 70 and 80 kDa, respectively (Figure 1, lanes 1 and 2).

Isolation of mutants affected in the hydroxamate–iron transport system

Strain CFBP1430 was mutagenized by random insertion of the Mu*d*IIpR13 genome. Around 1200 chloramphenicol-resistant clones were screened for alteration of their iron assimilation system based on changes in siderophore activity on CAS-agar medium. Three different phenotypes were identified: (i) colonies lacking a halo of decoloration (strains VD31, VD37 and VD61), (ii) colonies producing a weaker (strains VD41 and VD36) or (iii) a larger halo (strain VD17) than the wild-type. The three CAS-negative mutants as well as VD17 but not VD36 and VD41 failed to grow in the presence of EDDHA at a concentration allowing wild-type cells to grow well. CAS-negative mutants appeared to be also Csàky-negative while the mutants VD36 and VD41 were able to release hydroxamate in detectable amounts under limiting iron. Cross-feeding assays revealed that in

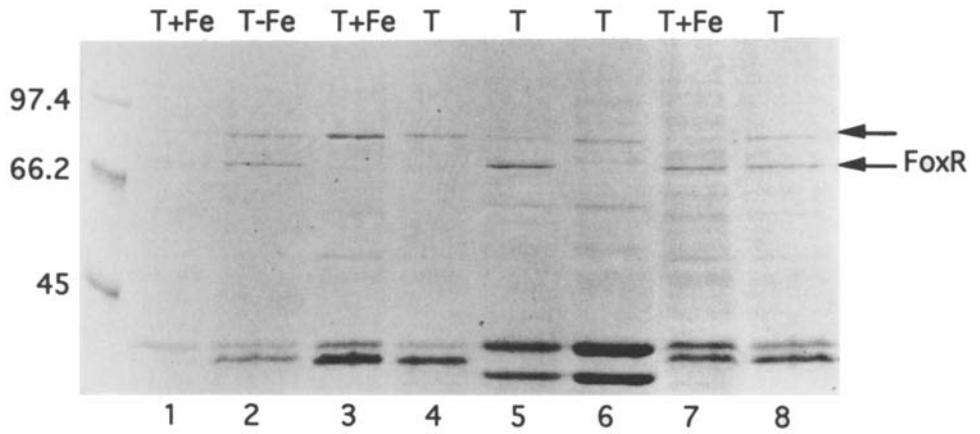


Figure 1. SDS–PAGE analysis of low-iron inducible outer membrane proteins of *E. amylovora*. Bacteria were grown up to an OD at 600 nm of 0.5–0.7 in Tris medium not supplemented (T) or supplemented with iron (T − Fe) or EDDHA (T − Fe) as specified in Material and methods. Strains were: lane 1 and 2, CFBP1430; 3 and 4, VD17; 5, MS172 p5G6; 6, MS172 pLA2917; 7 and 8, VD61. Low-iron repressible polypeptides are referred by an arrow. Standard proteins (SDS–PAGE low molecular mass standards from BioRad) are referred to by their molecular masses (in kDa).

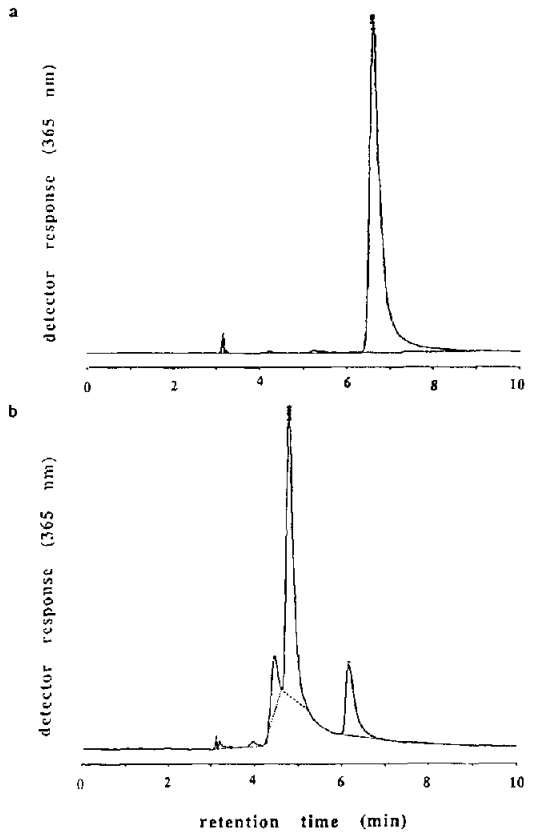


Figure 2. HPLC of purified ferrioxamine E (r.t. = 6.54 min) (**a**) and a mixture of ferrioxamines E, D_2 (r.t. = 4.76 min) and an additional cyclic ferrioxamine, X_1 or X_7 (r.t. = 4.44 min) (**b**) on a C_{18} reversed-phase column.

the presence of EDDHA, growth of the siderophore-deficient mutants was promoted with the parental strain, the siderophore-overproducing derivative VD17 or with ferrioxamine-containing culture supernatant fluids. These data strongly suggested that the strains VD31, VD57 and VD61 were deferrioxamine biosynthetic mutants, whereas no clear phenotype could be assigned to mutants VD36 and VD41. The mutant VD17 produced about three times more hydroxamate- and CAS-reacting material than the parental strain and failed to be cross-fed with any strain when grown in the presence of EDDHA. Since it was also unable to produce the iron repressible outer membrane protein of 70 kDa (Figure 1, lanes 3 and 4), we tentatively concluded that this protein was the ferrioxamine receptor in *E. amylovora*. Strain VD17 harbouring the mutation designated *foxR17* was chosen for isolation and purification of the siderophores.

The possibility that the mutants carry more than one copy of the Mud*II*pR13 genome within their chromosome was examined using Southern blot hybridization (data not shown). Mutants VD17, VD36, VD41 and VD61 contained a single insertion, whereas CAS-negative mutants VD31 and VD57 carry two mini-Mu copies. It is noteworthy that the frequency of double insertions identified in this case was much higher than that found for any other phenotype which has been screened for in this mutant library. It is possible that the desferrioxamine-negative phenotype may need two mutational events to occur.

Isolation and characterization of the E. amylovora *siderophores*

Siderophores were purified by solid-phase adsorption on XAD-2 (polystyrene) resin, followed by gel filtration over LH-20. Ferrioxamine E (Figure 4), the main constituent, was isolated by medium pressure chromatography on a silica gel column and its purity checked by HPLC (Figure 2a). It was identified by fast atom bombardment and electrospray mass spectrometry (Figure 3a), ^{1}H- and ^{13}C-NMR spectroscopy of the desferri form (Table 1), and comparison with published data (Huber 1984). Small amounts of ferrioxamine E analogs (Figure 4), like ferrioxamines D_2, an additional cyclic ferrioxamine, *m/z* 625 corresponding to X_1 or X_7 (Figures 2b and 3b) and linear analogue ferrioxamine G_1 (data not shown) were also detected by HPLC and electrospray

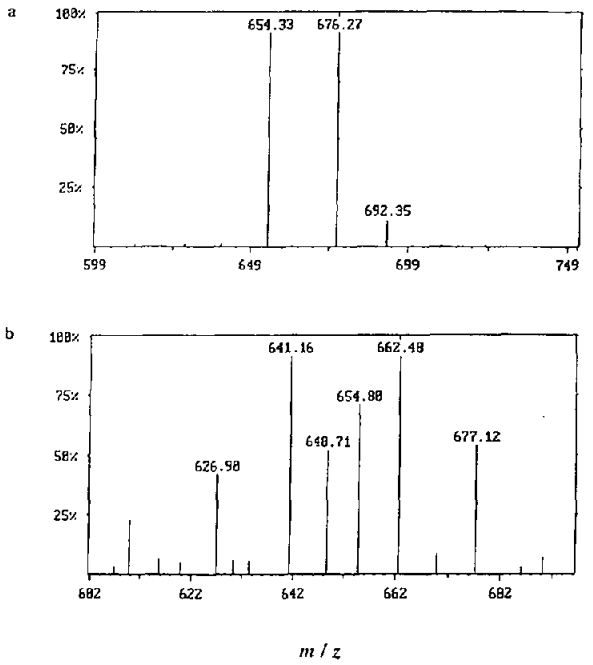
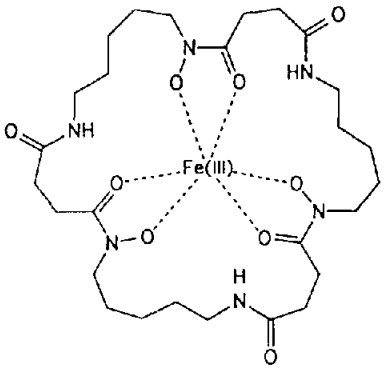


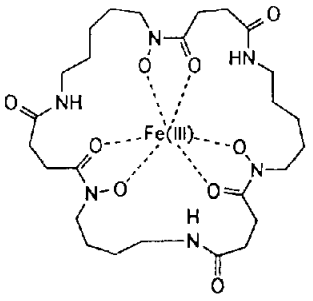
Figure 3. Electrospray mass spectra of purified ferrioxamine E (*m/z* 654 = MH^+; *m/z* 676 = MNa^+; *m/z* 692 = MK^+) (**a**) and a mixture of ferrioxamines E, D_2 (*m/z* 640 = MH^+; *m/z* 662 = MNa^+) and an additional cyclic ferrioxamine, X_1 or X_7 (*m/z* 626 = MH^+; *m/z* 648 = MNa^+) (**b**) isolated from *E. amylovora* mutant VD17.

Table 1. ^{13}C-NMR chemical shifts in D_2O of desferrioxamine E

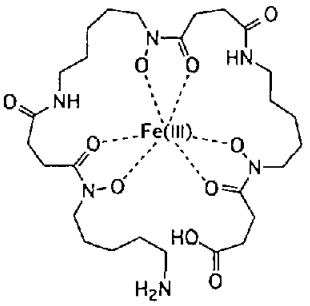
[-N(OH)-^{1}CH$_2$-^{2}CH$_2$-^{3}CH$_2$-^{4}CH$_2$-^{5}CH$_2$-NH-^{6}CO-^{7}CH$_2$-^{8}CH$_2$-^{9}CO-]$_3$										
^{n}C	9	6	1	5	7		4	8	2	3
$\delta_{p.p.m.}$	175.6	174.6	48.6	40.0	31.4		28.7	28.5	26.2	23.7



Ferrioxamine E



Ferrioxamine D_2



Ferrioxamine G_1

Figure 4. Structure of ferrioxamines E, D_2 and G_1, the main siderophores of *E. amylovora*.

mass spectrometry. No qualitative differences were found between the siderophore patterns of wild-type CFBP1430, siderophore weak producers VD36 and VD41 or siderophore-overproducing VD17. No ferrioxamine was detected in the supernatant fluids of strains VD31, VD57 and VD61 (this work and Feistner, personal communication). The corresponding dfo-deficient mutations were designated *dfo-31*, *dfo-57* and *dfo-61*, respectively.

Siderophore biological activity

Purified ferrioxamine E and D_2, together with other iron sources including ferrioxamine B and ferric citrate, were tested separately for their qualitative abilities to enhance the growth of siderophore non-producing mutants. The bioassay (data not shown) showed that ferrioxamines E and D_2 could reverse iron starvation of biosynthetic mutants but not of the transport mutant VD17, confirming that the purified compounds are actually biologically active siderophores which are specifically taken up by cells when loaded with iron. Growth stimulation characterized by distinct colonies surrounding the disks was also observed with ferrioxamine B which is not produced by *E. amylovora* cells. In contrast, citrate, aerobactin, chrysobactin, enterobactin, ferrichrome and rhodotulic acid could not supply iron to the siderophore non-producing cells.

Cloning of a parental genomic region restoring the transport mutation foxR17 *to normal*

In order to pick up the ferrioxamine receptor gene, we first cloned the host DNA sequence flanking the left terminal part of the MudIIpR13 prophage responsible for the transport mutation *foxR17*. The *Sal*I total DNA digest from the mutant strain was ligated into pUC19 DNA linearized with the same enzyme. After electroporation of DH5α cells, a few Cm^R, Ap^R transformant clones were recovered and their plasmid content analysed. The recombinant plasmid pAL1, carrying a host phage DNA insert of 9 kb in length, was identified (Figure 5). This DNA fragment was then used as a DNA–DNA hybridization probe to score the corresponding restriction fragment in the wild-type genomic DNA and to screen a wild-type gene library constructed in pLA2917. Thus, a 6.5 kb *Sal*I fragment was identified (Figure 5). This fragment was not present in its whole in the *Sal*I digest of the two cosmids detected with the probe. These recombinant cosmids designated p5G6 and pAL2 (Figure 5) were shown to carry a genomic insert of 25 and 23.5 kb in length, respectively. The region homologous to the radioactive probe was found in both constructions to be adjacent to the vector DNA.

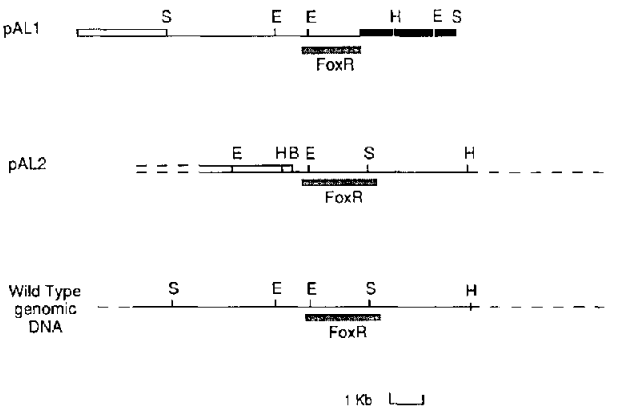


Figure 5. Physical map of the FoxR encoding genomic region of *E. amylovora*. In plasmid pAL1, the filled bar refers to the left part of the MudIIpR13 prophage responsible for the *foxR17* mutation and the open bar to pUC19 vector DNA. In cosmid pAL2, the open bar refers to pLA2917 DNA. The hatched box depicts the FoxR encoding locus.

Both cosmids were mobilized with the helper plasmid pRK2013 into strain VD17 carrying the mutation *foxR17*, using resistance to chloramphenicol as a counterselective marker. Several of the Tc^R transconjugants were streaked on CAS-agar medium. Formation of the halo was restored to normal and both types of transconjugants were able to grow in the presence of EDDHA. A bioassay indicated they had recovered the ability to utilize the ferrioxamine complexes as iron sources and SDS–PAGE analysis showed that the 70 kDa protein was produced in the outer membrane (Figure 1, lane 5). We concluded that the transport function missing in the mutant was encoded from a genomic region present in both cosmids.

Functional characterization of E. amylovora *recombinant cosmids in* E. coli

We determined whether the presence of the ferrioxamine receptor gene on the recombinant cosmids isolated was able to complement a $FhuE^-$ mutant of *E. coli*. Each of the two cosmids was mobilized into MS172 cells as described above. The resulting Tc^R transconjugants could grow on EDDHA when supplied exogenously with ferrioxamine B and E, unlike the parent carrying the cosmid vector alone. This indicated that these transconjugants could transport both ferrisiderophores via a specific cell surface receptor encoded by the genomic insert present on both cosmids. Analysis of their outer membrane profile evidenced the presence of an additional polypeptide of about 70 kDa produced under low as well as under high iron conditions, although in smaller amounts in the latter case. The lack of a tight control by iron in this case may be explained by the fact that the protein is encoded from a plasmid present in several copies per cell.

Discussion

The typical representatives of the genus *Erwinia* commonly referred to as the *amylovora* and *carotovora* groups are plant-related species including a majority of pathogens. *E. herbicola* species define a third and rather heterogeneous group which includes a number of bacteria associated with animals (Slade & Tiffin 1984). The few previous studies that attempted to characterize high-affinity iron transport systems in *Erwinia* have demonstrated a large structural heterogenity among the siderophores released by these bacteria under conditions of iron deficiency. This is in contrast to the more representative members of the *Enterobacteriaceae* which essentially are animal or human pathogens. Several *E. carotovora* strains including the subspecies *chrysanthemi* were shown to produce catechol-type siderophores, i.e. chrysobactin or other hitherto unidentified related compounds (Priou & Expert, unpublished data) and the hydroxamate type siderophore aerobactin (Ishimaru & Loper 1992). In *Pantoea agglomerans* (*E. herbicola* K4) a trihydro-samatex mediated iron uptake system using desferrioxamine E, D_2 and B as siderophores has been described (Berner & Winkelmann 1990, Matzanke *et al.* 1991). The present study shows that a ferrioxamine dependent system also operates in *E. amylovora* CFBP1430 and emphasizes the prevalence of desferrioxamines in *E. amylovora* (Feistner *et al.* 1993).

The ferrioxamine family of siderophores includes cyclic (bisucaberin and dfo E, D_2, $X_{1\ 2\ 7}$ and $T_{1\ 2\ 3}$) and linear (dfo $A_{1\ 2}$, B, C, F, $G_{1\ 2}$ and H) compounds (Bickel *et al.* 1960, Keller-Schierlein & Prelog 1962, Huber 1984, Fiedler *et al.* 1990, Feistner *et al.* 1993). Desferrioxamine E (nocardamin), the hydroxamate-type siderophore which has the highest complexation constant for ferric ion, has previously been isolated from actinomycetes (*Nocardia* and *Streptomyces* species) (Yang & Leong 1982, Müller & Raymond 1984) and other bacteria (*Chromobacteriaum violaceum*, *Pseudomonas stutzeri*, *Hafnia alvei* and *Erwinia herbicola*) (Müller & Zähner 1968, Meyer & Abdallah 1980, Berner *et al.* 1988, Reissbrodt *et al.* 1990). In addition to desferrioxamine E, *E. amylovora* produces small amounts of a series of structurally related compounds (this study and Feistner *et al.* 1993) which may be considered as minor metabolites. These diverse desferrioxamines must share a major step of their biosynthetic pathway, namely the decarboxylation of lysine as has been reported for *Streptomyces pilosus* (Schupp *et al.* 1988). The siderophore-deficient mutants described here likely lack one or several steps in this pathway, as based on their phenotypical traits. Characterization of the parental genomic region disrupted in the mutants will contribute to elucidating the biosynthetic functions.

In correlation with the induction of diverse desferrioxamines, *E. amylovora* cells respond to iron limitation by inducing two polypeptides of approximately 70 and 80 kDa, respectively, in the outer membrane. The 70 kDa protein which is lacking in strain VD17 altered in ferrioxamine transport was thus regarded as a good candidate for assuming the cognate receptor function FoxR. The cloning of a wild-type chromosomal region encoding the missing function of the mutant strain made it possible to functionally complement *E. coli* cells defective in FhuE, i.e. the coprogen receptor required also for ferrioxamine B transport, thus confirming our hypothesis. Likewise, the FoxR protein from *E. amylovora* can mediate the transport of ferrioxamine B, as shown in the bioassay with wild-type cells and *E. coli* transconjugants. It is worth noting here that the FhuE protein whose efficiency for ferrioxamine B transport is low, functions for ferrioxamine E. In addition, the FoxA receptor from *Pantoea agglomerans* recognizes different members of the ferrioxamine family including ferrioxamine E (Berner & Winkelmann 1990) and a receptor for ferrioxamine B and E has been identified in *Yersinia enterocolitica*. Interestingly, FoxA of *Y. enterocolitica* shares greater sequence homology with FhuA, the ferrichrome receptor, than with FhuE (Bäumler & Hantke 1992), thus validing the concept that other structural constraints than the strict specificity for the ferrisiderophore have prevailed during evolution in sequence conservation. From this standpoint and also considerations introduced above, a parallel analysis conducted on FoxR of *E. amylovora* seems to us very attractive. Finally, the failure of ferrioxamine-deficient *E. amylovora* cells to use the ferric complex of citrate further indicates that this bacterium does

not produce the cognate high-affinity uptake system as identified in *E. coli*, for instance (Hussein *et al.* 1981). This aspect might be of importance once the pathogen is invading the host plant, where ferric citrate represents the major iron source (Brown 1978). Neither were *E. amylovora* cells able to use other ferrisiderophores tested, indicating the lack of receptor function of the 80 kDa outer membrane protein for these iron carriers.

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