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Ferric Enterochelin Transport in *Yersinia enterocolitica*: Molecular and Evolutionary Aspects

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Yersinia enterocolitica **is well equipped for siderophore piracy, encompassing the utilization of siderophores such as ferrioxamine, ferrichrome, and ferrienterochelin. In this study, we report on the molecular and functional characterization of the *Yersinia fep-fes* gene cluster orthologous to the *Escherichia coli* ferrienterochelin transport genes (*fepA*, *fepDGC*, and *fepB*) and the esterase gene *fes*. In vitro transcription-translation analysis identified polypeptides of 30 and 35 kDa encoded by *fepC* and *fes*, respectively. A frameshift mutation within the *fepA* gene led to expression of a truncated polypeptide of 40 kDa. The *fepD*, *fepG*, and *fes* genes of *Y. enterocolitica* were shown to complement corresponding *E. coli* mutants. Insertional mutagenesis of *fepD* or *fes* genes abrogates enterochelin-supported growth of *Y. enterocolitica* on iron-chelated media. In contrast to *E. coli*, the *fep-fes* gene cluster in *Y. enterocolitica* consists solely of genes required for uptake and utilization of enterochelin (*fep*) and not of enterochelin synthesis genes such as *entF*. By Southern hybridization, *fepDGC* and *fes* sequences could be detected in *Y. enterocolitica* biotypes IB, IA, and II but not in biotype IV strains, *Yersinia pestis*, and *Yersinia pseudotuberculosis* strains. According to sequence alignment data and the coherent structure of the *Yersinia fep-fes* gene cluster, we suggest early genetic divergence of ferrienterochelin uptake determinants among species of the family *Enterobacteriaceae*.**

Acquisition of iron is essential for growth and survival of microorganisms. However, in neutral aqueous systems under aerobic conditions ferric iron (Fe^{3+}) forms insoluble oxyhydroxide compounds, resulting in extremely low concentrations of free Fe^{3+}. Similar conditions are met by microorganisms colonizing or invading vertebrate hosts where iron is tightly bound to host proteins such as transferrin, lactoferrin, or heme-containing proteins. For survival and multiplication in such an iron-restricted environment, facultative anaerobic microorganisms have developed high-affinity ferric iron uptake systems. Many cope with iron-deficient growth conditions by releasing small iron-chelating molecules termed siderophores, which subsequently can be taken up as ferric siderophores by specific transport systems (16, 33).

A large number of structurally diverse siderophores, which can be divided into three distinct major chemical classes, catecholates, hydroxamates, and heterocyclic compounds (e.g., pyochelin and yersiniabactin) (32), have been described.

Among the members of the family *Enterobacteriaceae*, the prototype of the catecholate siderophores, enterochelin (enterobactin), is widely distributed. Genes for enterochelin biosynthesis (*ent*), transport (*fep*), and the release of iron (ferric enterochelin esterase [*fes*]) are clustered. This enterochelin locus is about 20 kb in length and found in the genomes of *Escherichia coli*, *Salmonella enterica*, and *Shigella* species (15, 49, 58). The hydroxamate siderophore aerobactin is distributed with lower frequency among these three enterobacteria.

In contrast, pathogenic *Yersinia* species (*Yersinia pestis*, *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica*) do not produce catecholate or hydroxamate siderophores (5, 37, 42). However, they are endowed with siderophore uptake systems for catecholate siderophores (enterochelin) and hydroxamate siderophores (e.g., ferrioxamine and ferrichrome) (37). Moreover, the mouse-virulent (high-pathogenic) *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* biogroup 1B strains are able to produce and utilize the unique heterocyclic siderophore yersiniabactin (Ybt) (6, 10, 19, 23, 24, 34, 38, 39). The yersiniabactin biosynthesis and transport genes are clustered within a 45-kb region of the genome referred to as a high-pathogenicity island (6, 17, 18, 21, 34, 38). In addition to the Ybt system, pathogenic yersiniae carry a gene cluster involved in the uptake of heme-containing compounds (56).

So far, little is known about catecholate siderophore uptake in yersiniae. A putative outer membrane receptor for ferric enterochelin of about 90 kDa has been detected by monoclonal antibodies raised against *E. coli* ferric enterochelin receptor FepA (45). Moreover, a 60-kDa outer membrane protein has been identified as the receptor for a catechol-cephalosporin antibiotic (CccA) (5). In *E. coli*, catecholate siderophores are transported through the cytoplasmic membrane by means of an ATP binding cassette (ABC) transporter system (FepDGC) (12, 13). In the cytoplasm, ferric enterochelin is degraded to ferrous iron (Fe^{2+}) and 2,3-dihydroxybenzoyl serine derivatives by the esterase Fes. According to this observation, functional genes orthologous to *fes* and *fepDGC* may be present for the utilization of catecholate siderophores in yersiniae.

In order to identify genes involved in enterochelin uptake in yersiniae, we screened a genomic library of *Y. enterocolitica* serotype O8 for complementation of an *E. coli fes* mutant. We were able to identify a *Yersinia* gene cluster consisting of a set of genes (*fepB*, *fepDGC*, and *fes*) that reveal high identity to the corresponding genes of the enterochelin gene cluster of *E. coli*. In contrast to *E. coli*, the enterochelin locus of yersiniae does not carry genes involved in enterochelin biosynthesis (*ent*).

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The plasmids and bacterial strains used in this study are listed in Table 1. *E. coli* strains, except for the strains harboring the pGP1-2 plasmid, were grown in Luria broth (LB) or on LB agar plates at 37°C. *Yersinia* strains and *E. coli* strains harboring the pGP1-2 plasmid were cultivated at 28°C in the same medium (3). Blood agar plates were used for conjugation experiments. Antibiotics, when required, were included in the cul-

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TABLE 1. Bacterial strains and plasmids used in this study[a]

Strain or plasmid	Relevant characteristic(s)	Source or reference
Y. enterocolitica		
WA-314	Clinical isolate harboring the virulence plasmid pYVO8; Ybt^+	23
WA-314 *fes*	*fes* mutant derived from WA-314; Ybt^+	This study
WA-C	Plasmidless derivative of strain WA-314; Ybt^+	23
WA-Str	Streptomycin-resistant derivative of WA-C; Ybt^+	This study
Y1852	Derivative of WA-C; *fur* Ybt^+	24
WA-*fepD587*::Tn*552kan*	Derivative of WA-C; Tn*552kan* insertion in *fepD* gene; Ybt^+ Sm^r	This study
WA-*fes625*::Tn*552kan*	Derivative of WA-C; Tn*552kan* insertion in *fes* gene; Ybt^+ Sm^r	This study
Y-108-P	*Y. enterocolitica* serotype O3; clinical isolate; Ybt^-	23
Y-NF	*Y. enterocolitica* serotype O5; clinical isolate; Ybt^-	23
Y-5,27	*Y. enterocolitica* serotype O5.27; clinical isolate; Ybt^-	23
Y-96-P	*Y. enterocolitica* serotype O9; clinical isolate; Ybt^-	23
Y-O13	*Y. enterocolitica* serotype O13; clinical isolate; Ybt^+	23
Y-O20	*Y. enterocolitica* serotype O20; clinical isolate; Ybt^+	23
Y. pseudotuberculosis		
Y-P-I	Clinical isolate; serotype 1; Ybt^+	23
Y-P-II	Clinical isolate; serotype 2; Ybt^-	23
Y-P-III	Clinical isolate; serotype 3; Ybt^-	20
Y. pestis		
KUMA	Pgm^+ pYV^- pPCP1 Ybt^+	34
EV 76	Pgm^- pYV^+ pPCP1 Ybt^-	34
Other *Yersinia* spp.		
Y. frederiksenii		23
Y. intermedia		23
Y. kristensenii		23
Escherichia coli		
DH5α	*endA1 hsdR17* ($r_K^-m_K^+$) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1* Δ(*lacZYA-argF*)U169 (ϕ80*lacZ*ΔM15)	22
HB101	Δ(*gpt-proA*)*62 leuB6 thi-1 lacY1 hsdS*$_B$*20 recA rps* λ20(Str^r) *ara-14 galK2 xyl-5 mtl-1 supE44 mcrB*$_B$	9
WM1576	*thr leu lacY thi supE hsdR fhuA trxA* (pGP1-2)	57
AN272	*aroB fes proA argE pheA tyr trp*	29
AB1515.718	*purE42 proC14 leu-6 trpE38 thi-1 fhuA23 lacY1* (*fepD*::Tn*5*)	12
AB1515.764	*purE42 proC14 leu-6 trpE38 thi-1 fhuA23 lacY1* (*fepG*::Tn*5*)	12
AB1515.199	*purE42 proC14 leu-6 trpE38 thi-1 fhuA23 lacY1* (*fepC*::Tn*5*)	12
H1717	*aroB fhuF*::λplac Mu	55
S17-1 λ*pir*	*pir*$^+$ *tra*$^+$ Sm^r	52
Plasmids		
pACYC184	Cloning vector; Tc^r Cm^r	11
pBluescript KS(+)	Cloning vector; Ap^r	Stratagene
pT7-5	Vector for T7 protein expression; Ap^r	57
pGP1-2	Plasmid encoding T7 RNA polymerase; Km^r	57
pKAS-32	Suicide vector; Ap^r	53
pUC19	Cloning vector; Ap^r	
pAL101	pUC19 carrying Tn*552kan* transposon (1.1-kb *Spe*I fragment); Km^r Ap^r	30
pSI10	16-kb *Sau*3AI WA-C chromosomal DNA in pACYC184; Cm^r	This study
pB-1 and -2[b]	6.0-kb *Bgl*II fragment of pSI10 in pBluescript KS(+); *fes fepA**	This study
pH-1 and -2[b]	7.5-kb *Hin*dIII fragment of pSI10 in pBluescript KS(+); *fepDGC fes*	This study
pH-1C	3.5-kb *Cla*I/*Hin*dIII fragment of pH-1; *fepDGC*	This study
pHB3	3.3-kb *Bgl*II/*Hin*dIII fragment of pH-1; *fepDGC*	This study
pHB4	4.0-kb *Bgl*II/*Hin*dIII fragment of pH-1; *fes*	This study
pH-1*fes*::Tn*552kan*	pH-1 with Tn*552kan* insertion in *fes* gene; Km^r Ap^r	This study
pH-1*fepD*::Tn*552kan*	pH-1 with Tn*552kan* insertion in *fepD* gene; Km^r Ap^r	This study
pKAS*fes*::Tn*552kan*	5.3-kb *Eco*RI/*Kpn*I fragment of pH-1*fes*::Tn*552kan* in pKAS-32; Ap^r	This study
pKAS*fepD*::Tn*552kan*	3.3-kb *Bcl*I fragment of pH-1*fepD*::Tn*552kan* in pKAS-32; Ap^r	This study

[a] *Yersinia* strains producing the siderophore yersiniabactin have been designated Ybt^+, while those defective in yersiniabactin biosynthesis are Ybt^-.
[b] The inserts are cloned in both orientations.

ture media at the following concentrations: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; chloramphenicol, 30 μg/ml; and tetracycline, 15 μg/ml. Siderophore production was demonstrated on siderophore indicator colorimetric chromeazurol S (CAS) agar (50). For iron-deficient growth, strains were grown in NBD medium (nutrient broth plus 5 g of NaCl per liter and 200 μM 2,2′-dipyridyl). Siderophore feeding assays were performed as described elsewhere (24, 37, 43). With the *E. coli aroB* mutant strain AN272, the addition of 2,3-dihydroxybenzoic acid (2,3-DHBA; Sigma Chemical Co., St. Louis, Mo.) to a final concentration of 10 μg/ml was required.

Recombinant DNA methodology. DNA was isolated, digested with restriction endonucleases, and ligated by standard methods (3, 48) according to the recommendations of the manufacturers (Boehringer Mannheim Biochemicals, Pharmacia LKB, and New England BioLabs Ltd.). DNA fragments less than 10 kb were recovered from agarose gels with the GeneClean II kit (Bio 101, Inc., La Jolla, Calif.). *E. coli* DH5α was transformed by the $CaCl_2$ method (48), and *Y. enterocolitica* strains were transformed by electroporation (gene pulse apparatus; Bio-Rad Laboratories, Munich, Germany) according to the manufacturer's instructions. To generate the genomic library of *Y. enterocolitica* WA-C, genomic DNA was isolated by the sodium dodecyl sulfate-proteinase K method (35) and partially digested with endonuclease *Sau*3AI. After electrophoretic separation, DNA fragments of 6 to 20 kb were isolated by electroelution, purified further by phenol and chloroform extraction, and ligated into the *Bam*HI site of the pACYC184 vector (11). For hybridization, the restriction enzyme-digested genomic and plasmid DNA fragments were resolved through 0.8% agarose gels,

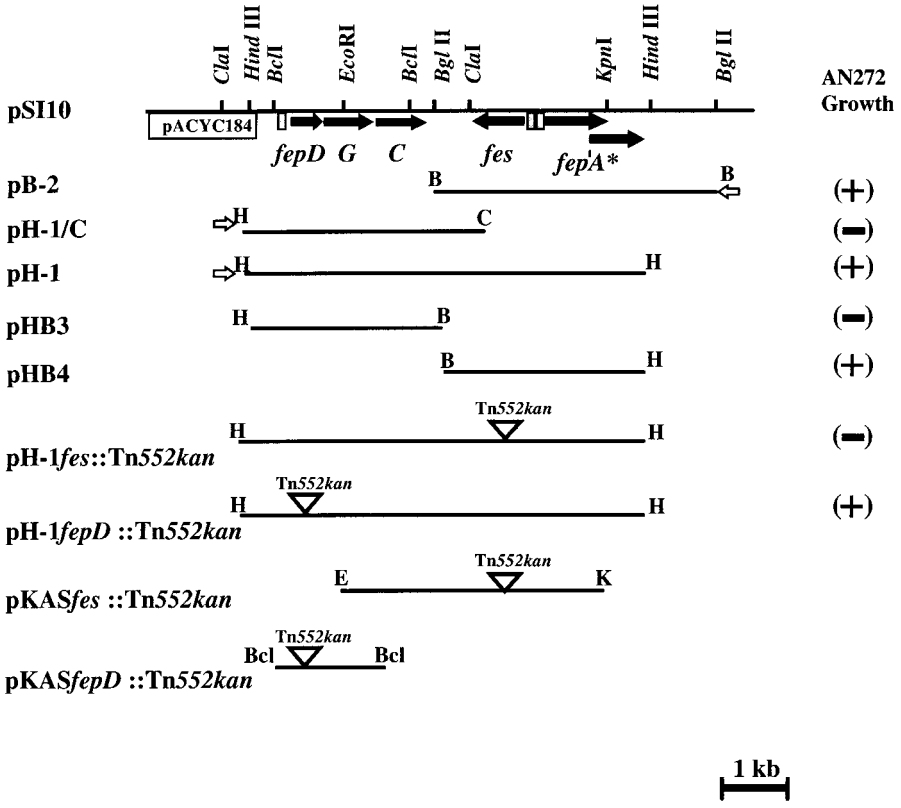

FIG. 1. Restriction map of pSI10 and various subclones used for protein expression and testing for complementation of growth of *E. coli* AN272. (+), plasmids which promote AN272 growth; (−), no growth stimulation. Arrows indicate the direction of transcription of genes included in the enterochelin uptake locus. Open inverted triangles represent sites of Tn*552kan* insertion. Open arrows represent the location of the T7 promoter. Grey shaded boxes upstream of *fepDCG*, *fes*, and *fepA** (frameshifted *fepA*) represent potential Fur boxes.

and DNA was transferred to Zeta-Probe BT blotting membranes (Bio-Rad Laboratories) according to the method of Southern (54). After prehybridization at 68°C for 2 h and addition of heat-denatured probe, blots were incubated overnight at 68°C in the absence of formamide. The detection was performed with the ECF Random-Prime labelling and detection system (Amersham Pharmacia Biotech, Freiburg, Germany) according to the manufacturer's instructions.

Protein analyses. Protein expression was determined according to the procedure of Tabor and Richardson (57). For this, the 7.5-kb *Hin*dIII fragment as well as the 6.0-kb *Bgl*II fragment of the pSI10 plasmid was cloned in both orientations into the pBluescript KS(+) vector, generating the recombinant plasmids pH-1/pH-2 and pB-1/pB-2, respectively (Table 1 and Fig. 1). These plasmids were transformed into *E. coli* WM1576 carrying pGP1-2, a T7 RNA polymerase-encoding plasmid. Proteins were radiolabelled with 10 μCi of [^{35}S]methionine (ICN Biomedicals GmbH, Eschwege, Germany), cells were treated at 100°C for 10 min in sample buffer (60 mM Tris-HCl [pH 6.8], 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10% [vol/vol] glycerol, 0.001% bromophenol blue), and proteins were separated by electrophoresis on polyacrylamide gels (3, 28). Dried gels were exposed to Kodak Bio Max MR film at room temperature.

Nucleotide sequencing. In order to facilitate sequencing of the *fepA*-, *fepDGC*-, and *fes*-homologous genes of *Y. enterocolitica* O8 strain WA-C, a series of subclones of the plasmids pH-1 and pH-2, containing progressively smaller portions of the original inserts, were obtained by combined exonuclease III-S1 nuclease treatment (nested deletion kit; Amersham). The generated sets of smaller fragments were purified with anion-exchange resin columns (Qiagen, Hilden, Germany), and the nucleotide sequences of both strands were determined by the TaqDyeDideoxy terminator method with the ABI model 373A DNA sequencer (Applied Biosystems, Weiterstadt, Germany). Sequence analysis was performed with ANALYSIS software (version 1.2.0; Applied Biosystems), MacDNASIS software (version 2.0; Hitachi Software Engineering Co., Tokyo, Japan), and DNAMAN sequence analysis software (Lynnon BioSoft, Vandrevil, Quebec, Canada). The nucleotide sequences were compared to those in SWISSPROT, Pir, and GenPept at the National Center for Biotechnology Information by using the program blastX and to GenBank and EMBL by using the program blastN (1).

Transposon (Tn*552kan*) mutagenesis of *fes* and *fepDGC* genes. The pH-1 plasmid covering the *fepD* and *fes* genes (Fig. 1 and 2) was used as a target for in vitro transposon mutagenesis. Tn*552kan* carrying a kanamycin cassette (kindly provided by Tom Griffin, Yale University) was inserted into these genes, thereby generating plasmids pH-1*fepD*::Tn*552kan* and pH-1*fes*::Tn*552kan*, respectively (30, 44). The insertion of Tn*552kan* was verified by PCR and sequencing. Subcloning of DNA fragments carrying Tn*552kan* targeted *fepD* and *fes* into the suicide vector pKAS-32, which gave rise to plasmids pKAS*fepD*::Tn*552kan* and pKAS*fes*::Tn*552kan*, respectively (53). *E. coli* S17-1 λ*pir* was used for propagation of all suicide vector constructs and as a donor for introduction of these constructs into *Y. enterocolitica* O8 strain WA-Str. Chromosomal DNA of mutants was routinely tested by Southern hybridization with suitable DNA probes to confirm insertional inactivation of genes of interest.

PCR analyses. Genomic DNA (50 pg) or 1 μl of cells (10^4 to 10^5 CFU) of the *Yersinia* strains listed in Table 1 was used as a template in PCRs along with oligonucleotides (Metabion, Munich, Germany; Roth, Karlsruhe, Germany) as described below. The amplification mixtures consisted of either *Taq* DNA polymerase (ABI/Perkin-Elmer, Weiterstadt, Germany) or *Pfu* DNA polymerase (Stratagene, Heidelberg, Germany), 200 μM deoxynucleoside triphosphates, 1.5 μM $MgCl_2$, and 0.4 μM primers. All PCRs were carried out in a GeneAmp PCR System 9700 thermal cycler (Perkin-Elmer). Products from these reactions were resolved by agarose gel electrophoresis. The initial denaturation step (94°C, 5 min) was followed by 30 cycles of denaturation (94°C, 1 min), annealing (annealing temperature [T_m], 1 min), and extension (72°C, 1 min) with one final extension step (72°C, 8 min). Sequences of the forward primers (FP) and reverse primers (RP) used for PCRs, the sizes of the amplified fragments (S), and the annealing temperatures (T_m) were as follows: (i) *fepD*.for (FP), 5′-GTG TGA TTG CCT TAC TAT TG-3′; *fepD*.rev (RP), 5′-CGG TCA TCC TTT TAT TAC GG-3′ (S, 397 bp; T_m, 55°C); (ii) *fes*.for (FP), 5′-GCC GGC AGG CAC AGC GTA AT-3′; *fes*.rev (RP), 5′-GGC CAA CCC ACC CAA AAC TT-3′ (S, 562 bp; T_m, 58°C); (iii) *fepA*.for (FP), 5′-TAC GCC AAA ATA CCT TAC GAT-3′; *fepA*.rev (RP), 5′-TGT AAA TAC ACC CCC ACC TGA-3′; (S, 438 bp; T_m, 56°C).

In order to determine the DNA region upstream of *fepDGC* (Fig. 2), the LA-PCR in vitro cloning technique (Takara Shuzo Co., Ltd., Kioto, Japan) was used. In brief, DNA linkers were ligated to chromosomal DNA of strain WA-C cleaved with *Eco*RI. Following ligation, a nested PCR was performed with primers derived from the linker together with primers derived from the *fepD* gene (S1.*fepD*, 5′-GGC GGG CTT CAT AGT GCG GTC ATC CTT TTA-3′; S2.*fepD*, 5′-GCC CGC CGC CCT GAG TTC CTA CCC AAT ACA-3′). The

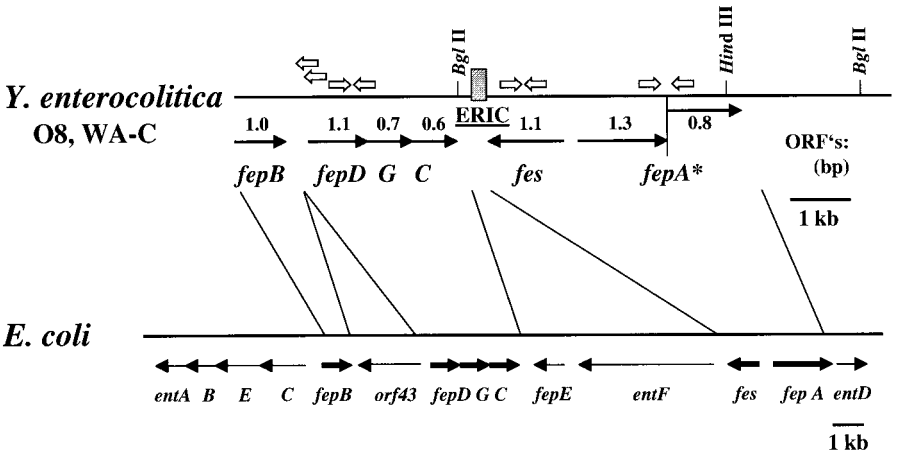

FIG. 2. Comparison of the enterochelin gene cluster of *Y. enterocolitica* O8 strain WA-C to the *E. coli* gene cluster encoding proteins involved in biosynthesis and transport of enterochelin. Open arrows indicate the locations of primers used for RT-PCR and LA-PCR cloning procedures. The shaded box between *fes* and *fepC* represents the location of the ERIC sequence in *Y. enterocolitica*.

nucleotide sequence of a resulting 2-kb PCR fragment covering *Yersinia fepB* was determined.

Isolation of total RNA and RT-PCR. *Y. enterocolitica* WA-C was grown in LB medium at 26°C to an optical density at 600 nm (OD_{600}) of 1.0. After centrifugation of 1 ml of the culture, the pellet was treated with the RNA Easy kit (Promega, Heidelberg, Germany) according to the manufacturer's instructions. The RNA was dissolved in 50 μl of RNase-free water and stored at −70°C. For grown bacteria, several independent reverse transcriptase PCRs (RT-PCRs) were performed. As an initial step, contaminating DNA was digested by incubation with 2 U of RNase-free DNase (Promega) for 30 min at 48°C, DNase was heat inactivated at 70°C for 10 min, and the RNA concentration was determined spectrophotometrically. Ten nanograms of the total RNA was subsequently subjected to RT-PCR with primers listed above. For RT-PCR, the Access RT-PCR system (Promega) was used as recommended by the manufacturer. Controls consisting of reaction mixtures with RNA preparations without cDNA synthesis steps were tested with each primer pair. Products were analyzed by loading 20 μl of the PCR mixture into adjacent wells of a 1% agarose gel (Fig. 3).

FURTA. The Fur titration assay (FURTA) was performed as described previously (55). In brief, plasmid pSI10 was double digested with the restriction enzymes *Hin*dIII and *Bgl*II. A 3.3-kb *Hin*dIII/*Bgl*II fragment, containing the putative Fur box of the *fepDGC* operon, and a 4.0-kb *Hin*dIII/*Bgl*II fragment, containing the Fur boxes of the *fepA* and *fes* genes, were subcloned in pBluescript KS(+) vector, resulting in plasmids pHB3 and pHB4, respectively (Fig. 1). These plasmids, along with the pBluescript KS(+) vector as negative control, were transformed into *E. coli* H1717. Transformants were plated on MacConkey agar and evaluated for Lac^+ phenotype (55).

Isolation of enterochelin and enterochelin feeding bioassay. Enterochelin was isolated as described by Langman et al. (29). Briefly, supernatant from the enterochelin-hyperproducing *E. coli* strain AN311 was acidified with H_2SO_4 and extracted with ethyl acetate. After washing with sodium phosphate buffer in order to remove enterochelin by-products such as 2,3-dihydroxybenzoyl serine, the enterochelin-containing ethyl acetate fraction was concentrated by rotary evaporation (crude enterochelin).

The strains to be tested were grown in NB medium (8 g of nutrient broth and 5 g of NaCl per 1 liter of distilled water) to an OD_{600} of 0.5. Thirty microliters of the bacteria was seeded in 10 ml of 0.6% H_2O top agar on 1% NB agar, both containing the iron chelator 2,2′-dipyridyl at a concentration of 200 μM (24). Filter disks impregnated with 10 μl of a methanolic solution of enterochelin were used. The crude enterochelin solution was adjusted to an OD_{578} of 0.1 (37). The diameters of the zone of enhanced bacterial growth around the filter paper were determined after 24 h of culture at 26°C (*Yersinia*) and 37°C (*E. coli*).

Nucleotide sequence accession number. The nucleotide sequences of the *fes* and *fepDGC* genes have been deposited in the GenBank database and assigned the accession no. U41370 and AF082879, respectively.

RESULTS

Identification of a *Yersinia fes* homologue by genetic complementation. To identify a ferric enterochelin siderophore uptake system of *Y. enterocolitica*, a genomic library derived from *Y. enterocolitica* O8 strain WA-C was introduced into *E. coli* AN272 (*fes aroB*), a mutant defective in the synthesis of the enterochelin esterase Fes. The recombinant clones were selected for *fes* complementation on NBD medium containing DHBA. The addition of 2,3-DHBA was required for enterochelin biosynthesis of AN272. Ten representative colonies were chosen for plasmid extraction. All of these clones carried a unique plasmid designated pSI10 (Fig. 1). By using different combinations of restriction enzymes, a physical map of pSI10 was determined (Fig. 1). A 7.5-kb *Hin*dIII fragment of pSI10 was shown to restore the *fes* mutation of *E. coli* AN272.

Sequence analysis of the *Y. enterocolitica* O8 *fes* gene. For further characterization, the 7.5-kb *Hin*dIII fragment was subcloned into the vector pBluescript KS(+) in both orientations and designated pH-1 and pH-2. Sequencing of the entire 7.5-kb *Hin*dIII fragment revealed a single open reading frame (ORF) of 1,059 bp, showing 54% identity to the *fes* gene of *E. coli* (Fig. 2). Upstream of this ORF, a putative promoter region comprising a sequence with 68% identity to the *E. coli* Fur-binding consensus sequence (FBS) was found (Table 2) (14). A putative Shine-Dalgarno sequence is located upstream of the initiating methionine codon. Moreover, an imperfect inverted repeat is located beyond the translational stop codon and may serve as a transcriptional terminator. The deduced amino acid sequence, encoded by the 1,059-bp ORF, was an-

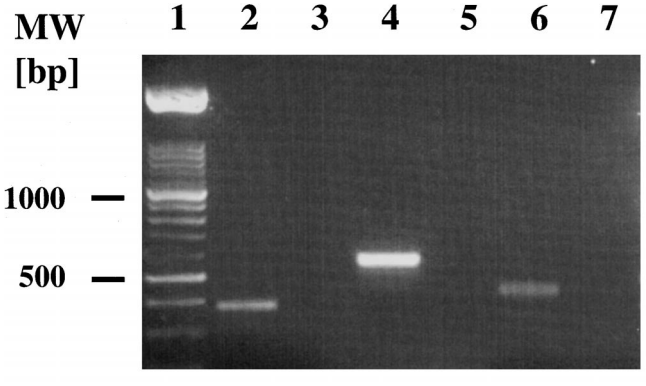

FIG. 3. Detection of the transcription of *fepD*-, *fes*-, and *fepA*-homologous genes of *Y. enterocolitica* by RT-PCR. Lane 1, DNA molecular size (MW) markers; lanes 2 and 3, *fepD*; lanes 4 and 5, *fes*; lanes 6 and 7, *fepA*-homologous gene. PCR mixtures in lanes 2, 4, and 6 contained RT, whereas mixtures in lanes 3, 5, and 7 lacked RT and served as negative controls.

TABLE 2. Comparison of the Fur boxes from *fes*, *fepDGC*, and *fepA* of *Y. enterocolitica* O8 strain WA-C to the *E. coli* Fur box consensus sequence (FBS)

Fur box	Sequence[a]	% Identity
Yersinia fes	5′ **GCAAATAATAATACTTCTC** 3′	68.4
Yersinia fepA	5′ **GATAATAATAA**ATC**T**GGGT 3′	57.9
Yersinia fepDGC	5′ **AATAATGATAATCA**AAA**C**T 3′	73.7
Fur box consensus sequence	5′ GATAATGATAATCATTATC 3′	

[a] Nucleotides identical to those in the Fur box consensus sequence are indicated by bold type.

alyzed for homologous proteins in the Swiss-Prot database and was found to be highly homologous to the *E. coli* Fes sequence with 40% identity and 56% similarity over a stretch of 293 amino acids as well as 37% identity and 57% similarity to Fes of *Erwinia chrysanthemi*. The putative *Yersinia* Fes consists of 353 amino acids and has a calculated size of 39,837 Da.

A *fepA* homologue carrying a frameshift mutation. Upstream of *fes*, two overlapping ORFs with a total size of 2,175 bp, exhibiting sequence identity to the 5′ and 3′ halves of the *E. coli* enterochelin receptor gene *fepA*, respectively, were identified. Upstream of the first ORF, a potential promoter was found, overlapped by a sequence revealing identity to the *E. coli* FBS (Table 2). From this, we presume a single-frameshift deletion within the initially intact *fepA* gene. The putative frameshift deletion in the *fepA*-homologous gene was confirmed by sequencing of PCR products of chromosomal DNA of *Y. enterocolitica* O8 strain WA-C.

In *E. coli*, the enterochelin gene cluster is terminated by the *entD* gene that is located immediately downstream of *fepA* (Fig. 2). The *entD* gene encodes an enzyme involved in the biosynthesis of enterochelin. In *E. coli*, the *entD* gene contains a high frequency of rare codons and is downstream of two repetitive extragenic palindromic sequences, suggesting chromosomal rearrangements in this region. In *Y. enterocolitica*, however, no *entD*-homologous gene is found at the corresponding position downstream of *fepA*.

Enterochelin transport proteins are encoded downstream of *fes*. Downstream of *fes*, in an opposite orientation a set of three ORFs (Fig. 1 and 2) forming a putative Fur-regulated operon as indicated by a preceding FBS was identified (Table 2). The nucleotide sequences of these three ORFs were found to be homologous to the *E. coli* genes *fepD*, *-G*, and *-C*, which encode proteins involved in ferric enterochelin transport (Fig. 2). Accordingly, the deduced amino acid sequence of the polypeptides encoded by the *Yersinia* homologous *fepDGC* genes revealed striking similarity to the set of integral membrane proteins involved in iron transport through high-affinity periplasmic transport systems (*Yersinia-E. coli* FepD, 51% identity and 73% similarity over 315 amino acids; *Yersinia-E. coli* FepG, 65% identity and 78% similarity over 246 amino acids). Hydropathy profiles of the predicted proteins *Yersinia* FepD and *Yersinia* FepG indicate that these are extremely hydrophobic proteins with eight and six predicted transmembrane helices, respectively (31). The deduced amino acid sequence of the *Yersinia* FepC homologue shows a strong identity to highly conserved regions of peripheral membrane ATP-binding proteins. A consensus sequence has been determined for both the amino- and the carboxy-terminal regions of nucleotide-binding proteins (51), with the predicted *Yersinia* FepC sequence containing 11 of 12 amino acids of the first region and 10 of 11 of the second region, including the completely conserved GKS (Walker motif A) and DEP (Walker motif B) amino acid sequence motifs (25, 59). The presence of a consensus nucleotide-binding site suggests FepC-homologous function in *Yersinia*. In order to obtain sequence information on the region upstream of *fepDGC* which is not located on the recombinant plasmid pSI10, the LA-PCR cloning method was used. Thus, upstream of *fepDGC* we found an ORF homologous to *fepB*. The deduced putative polypeptide has 59% identity and 75% similarity with the periplasmic binding protein FepB of *E. coli* (Fig. 2). In contrast to the enterochelin cluster of *E. coli*, no *orf43*-homologous gene could be detected in *Y. enterocolitica* (Fig. 2).

An ERIC sequence is located within the *fep-fes* gene cluster of *Y. enterocolitica* O8. Sequencing of the DNA region between *Yersinia fepC* and *fes* revealed a 126-bp nucleotide sequence with extensive (81%) identity to the enterobacterial repetitive intergenic consensus (ERIC) sequence (27) (Fig. 2 and 4). It has 84% identity to another ERIC sequence of *Y. enterocolitica* O8 strain WA-314 found upstream of the *ybtA* gene of the yersiniabactin siderophore gene cluster (41). As is common for ERIC sequences, this element is located in an intergenic region and shares a core inverted repeat (Fig. 4) which could potentially form a stem-loop structure. The sequence does not resemble any known insertion sequence or transposable element. Interestingly, compared to the enterochelin gene cluster of *E. coli*, the ERIC sequence in the *Y. enterocolitica* chromosome is located at the position where orthologous *entF* and *fepE* genes are expected (Fig. 2). Thus, the presence of an ERIC sequence at this position may suggest that the biosynthesis genes have been deleted in *Yersinia*.

Identification of polypeptides encoded by the *fes-fep* gene cluster of *Y. enterocolitica* O8. The transcription of *fepDGC* as well as of *Yersinia fes* and *fepA* was confirmed by RT-PCR. Amplification products of expected sizes were obtained in those samples containing RT whereas samples without RT revealed no PCR product (Fig. 3). This indicated that all three genes were transcribed in *Y. enterocolitica* O8 strain WA-C.

To identify polypeptides encoded by the *Yersinia fes-fep* gene cluster, the T7-based expression system of Tabor and Richardson was used (57). Appropriate restriction subfragments of the pSI10 plasmid were subcloned downstream of the T7 promoter of pBluescript KS(+), resulting in plasmids pH-1, pH-1/C, and pB-2, respectively (Fig. 1). For [^{35}S]methionine labelling, these plasmids were introduced into *E. coli* WM1576 carrying the gene for T7 RNA polymerase. Expression of the *fepDGC* operon (pH-1C plasmid) resulted in a 30-kDa polypeptide.

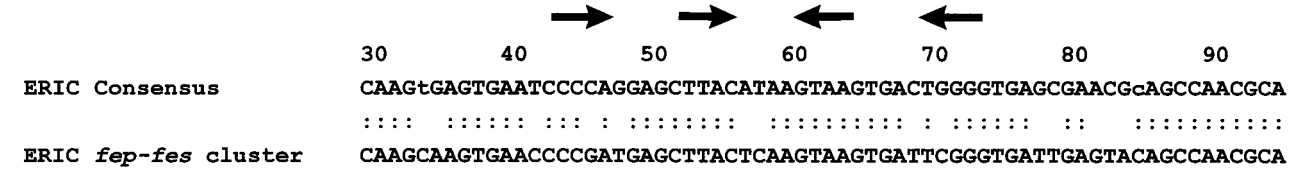

FIG. 4. Comparison of the ERIC sequence from the *Y. enterocolitica fes-fepDGC* DNA region with the consensus (27). The core inverted repeats found in all ERIC sequences are indicated by the arrows.

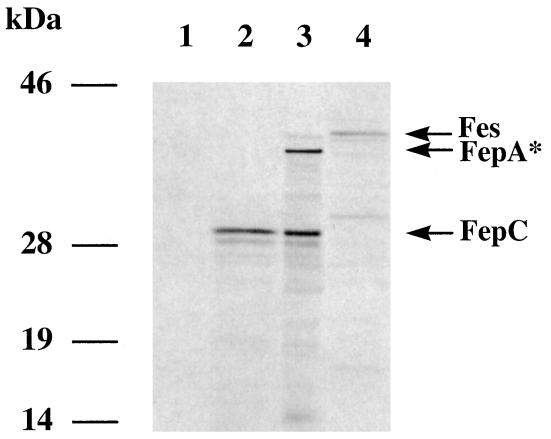

FIG. 5. T7 protein expression assay with *E. coli* WM1576 harboring pGP1-2 and other plasmids. Shown are autoradiograms of plasmid-encoded proteins labelled with ^{35}S-amino acids. Lane 1, pACYC184; lane 2, pH-1/C; lane 3, pH-1; lane 4, pB-2. Arrows indicate the presumed expression products: Fes, truncated FepA*, and FepC.

The molecular weight of this polypeptide corresponds closely to that of FepC of *E. coli* (51). It was not possible to detect FepD and FepG by T7 expression. This has already been reported for *E. coli* FepD and FepG and is probably due to the hydrophobic character of these proteins (51). The expression of genes from the entire 7.5-kb *Hin*dIII fragment (pH-1) reveals an additional polypeptide of about 35 kDa. This size corresponds closely to the predicted truncated polypeptide of the *Yersinia* FepA (Fig. 1 and 5). According to the orientation of T7 transcription, the *fes* gene product was not expressed by using pH-1 and pH-1C. Therefore, plasmid pB-2 was subjected to T7-based expression, revealing synthesis of a protein with an estimated molecular mass of 40 kDa, which is similar to the size of the known *E. coli* Fes protein (Fig. 2).

Functional analysis of the *Y. enterocolitica fes-fep* gene cluster. To determine the role of the *Y. enterocolitica fes-fep* gene cluster in utilization of catecholate siderophores, we investigated the ability of *Yersinia fes-fep* genes to complement orthologous *E. coli* mutants. For this purpose, *Yersinia fes* and *fepD* genes of plasmid pH-1 were inactivated by Tn*552kan* insertion, resulting in pH-1*fes*::Tn*552kan* and pH-1*fepD*::Tn*552kan* plasmids. pH-1*fepD*::Tn*552kan* and pH-1 were introduced into *E. coli fepD* and *fepG* mutant strains (AB1515.718 and AB1515.764, respectively). The pH-1 and pH-1*fes*::Tn*552kan* plasmids were transferred into *E. coli fes* mutant strain AN272. All transformants were tested for enterochelin-supported growth on NBD agar plates supplemented with 100 μM 2,2′-dipyridyl (Table 3). The pH-1 plasmid conferred enterochelin-supported growth on all the *E. coli* mutants. By introduction of plasmids carrying insertionally inactivated *Yersinia fes* or *fepD* (pH-1*fes*::Tn*552kan* or pH-1*fepD*::Tn*552kan*), it was not possible to complement the corresponding *E. coli* mutant. Interestingly, under higher iron-chelating conditions (200 μM 2,2′-dipyridyl) only *E. coli* AN272 *fes* carrying pH-1 showed enterochelin-supported growth. Obviously, complementation of corresponding *E. coli* mutants with orthologous *fepD* and *fepG* was less efficient than that with orthologous *Yersinia fes*. Presumably, *Yersinia* FepD and FepG are not optimal partners for the corresponding *E. coli* Fep proteins to form a highly efficient enterochelin transport system.

According to this assumption, we expected that coexpression of *Yersinia fepDGC* and *E. coli fepDGC* in *E. coli* should result in mixed FepDGC transport complexes with impaired ferric enterochelin utilization and consequently in enterochelin hyperproduction. On the other hand, it is also conceivable that the periplasmic catecholate siderophore binding protein FepB of *E. coli* does not interact properly with the *Yersinia* FepDGC complex. In fact, *E. coli* DH5α harboring plasmid pH-1 produced much larger haloes on CAS agar (CAS^{++} phenotype) than the parental strain (CAS^{+} phenotype), indicating enhanced enterochelin production. Progressively truncated subfragments of the 7.5-kb *Hin*dIII fragment of pH-1 were obtained by exonuclease treatment. By subcloning these subfragments in DH5α, we found that the CAS^{++} phenotype was dependent on the integrity solely of the *Yersinia fepDGC* operon. Thus, a Fur capture phenomenon caused by introduction of *Yersinia* Fur boxes on the recombinant plasmid into *E. coli* DH5α could largely be excluded.

However, the deduced Fur boxes located on the *Y. enterocolitica fes-fep* gene cluster are functional in *E. coli*. This could be demonstrated for plasmid pHB3 (carrying promoters of *fes* and *fepA*) and for plasmid pHB4 (*fepDGC* promoter) by using the FURTA (reference 55 and data not shown).

Construction of *Yersinia* mutants defective in *fepD* and *fes*. To determine the role of *fes* and *fepDGC* in catecholate siderophore uptake in *Y. enterocolitica*, we constructed *fes* and *fepD* mutants by allelic exchange with the suicide plasmids pKAS*fes*::Tn*552kan* and pKAS*fepD*::Tn*552kan* (Fig. 1). The resulting mutants WA-*fes625*::Tn*552kan* and WA-*fepD587*::Tn*552kan* together with the parental strain WA-C were tested for enterochelin utilization by a bioassay as described elsewhere (37). In contrast to strain WA-C, the growth of the *fepD* and *fes* mutant strains was not supported by applying enterochelin-soaked filter disks. However, enterochelin-supported growth became evident after introduction of plasmid pH-1 into the mutant strains. Thus, the *Yersinia fes-fep* gene cluster is involved in ferric enterochelin siderophore transport and utilization.

Distribution of the *Y. enterocolitica fes-fep* gene cluster among different *Yersinia* species. In order to investigate the distribution of the *fes* gene among different serovars of *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*, as well as among nonpathogenic *Yersinia* species (*Yersinia frederiksenii*, *Yersinia intermedia*, and *Yersinia kristensenii*), Southern hybridizations were performed with a PCR-derived *fes* gene probe. Hybridization of *Cla*I-digested genomic DNA (Fig. 6) showed that the *Yersinia fes* gene is detectable in all *Y. enterocolitica* serovars pathogenic to humans except serovar O3 (14 strains of serovar O3 were tested [47]). However, the *fes* gene is absent in *Y.*

TABLE 3. Complementation of *E. coli* mutants of ferric enterochelin esterase (AN272 *fes*) and ferric enterochelin permease (AB1515.718 *fepD* and AB1515.764 *fepG*)[a]

Plasmid used for complementation	Complementation of mutant at concn (μM) of 2,2′-dipyridyl		
	AN272 *fes*, 200	AB1515.718 *fepD* and AB1515.764 *fepG*	
		100	200
pACYC184	ϕ	ϕ	ϕ
pBluescript KS(+)	ϕ	ϕ	ϕ
pSI10	+	+	ϕ
pH-1	+	+	ϕ
pH-1*fes*::Tn*552kan*	ϕ	ND	ND
pH-1*fepD*::Tn*552kan*	ND	ϕ	ϕ

[a] Lack of complementation (ϕ) or positive complementation (+) by the bioassay as described in Materials and Methods. ND, not determined.

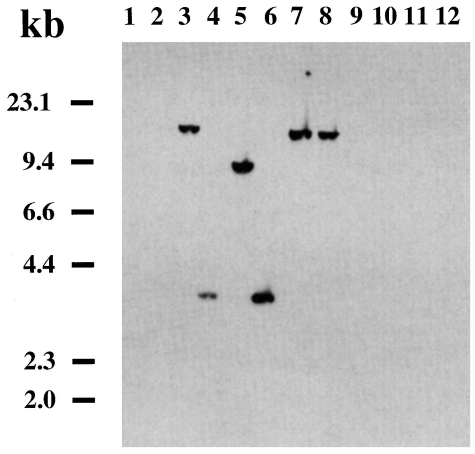

FIG. 6. Southern blotting of *Cla*I-digested chromosomal DNA of different *Y. enterocolitica* serovars and *E. coli* DH5α. The preparation was probed with a labelled PCR product generated from the *Yersinia fes* gene. Lane 1, *E. coli* DH5α; lane 2, *Y. enterocolitica* O3; lane 3, O5; lane 4, O5.27; lane 5, O8; lane 6, O9; lane 7, O13; lane 8, O20; lane 9, *Y. pseudotuberculosis* I; lane 10, *Y. pseudotuberculosis* II; lane 11, *Y. pseudotuberculosis* III; lane 12, *Y. pestis pgm*$^+$.

pseudotuberculosis serovars 1, 2, and 3; *Y. pestis* KUMA; and the nonpathogenic *Yersinia* species. In *Y. enterocolitica* O8, the *fes* probe reacted with a fragment of about 9 kb; in serovars O5, O13, and O20, a hybridizing band of approximately 18 kb was detected. Serovars O5.27 and O9 revealed a hybridizing band of about 3 kb. Corresponding results were observed for *Cla*I-digested genomic DNA with a probe derived from *Yersinia fepD* and *fepA* genes, indicating that *fes*-positive strains were also positive for *fepD* and *fepA* (data not shown). The conservation of the *fes* gene in different *Y. enterocolitica* serovars was determined by sequencing of PCR fragments covering the *fes* gene. Identity of 98.6 to 100% was found for the *Yersinia fes* gene. However, the base pair exchanges observed within the *fes* genes have no impact on changes of the predicted amino acid sequence.

As shown above, the *fepA* gene of *Y. enterocolitica* O8 appears to be interrupted by a frameshift. To investigate the presence of the frameshifted *fepA* gene among other *Y. enterocolitica* serovars, suitable primers were designed to amplify the central part of *fepA* by PCR. Only *Y. enterocolitica* O20, O5, and O13 yielded abundant PCR products which were subsequently sequenced. The serotype O20 strain carries an identical frameshift deletion of the *fepA* homologue. Interestingly, sequencing of the corresponding gene in *Y. enterocolitica* serotype O5 and O13 strains revealed a 31-bp insertion of DNA within this region leading to a single ORF. However, the significance of the *fepA* frameshift mutation remains to be clarified.

DISCUSSION

The objective of this study was to characterize the ferric enterochelin uptake and utilization of *Y. enterocolitica* serotype O8. For this purpose, we used an *E. coli fes* mutant as the recipient of a *Yersinia* genomic library and selected for *fes* complementation. We were able to isolate an ~7.5-kb fragment of genomic DNA from *Y. enterocolitica* O8 composed of five genes arranged in three distinct transcriptional units (Fig. 1 and 2). The first unit consists of functional genes highly homologous to the *E. coli fepDGC* genes. This operon encodes three polypeptides which collectively resemble a cytoplasmic membrane transport system for ferric enterochelin belonging to the superfamily of ABC transporters (12, 25, 51). FepC has signature ATP-binding motifs (25, 59), while FepD and FepG are endowed with characteristics of integral membrane permeases (51). By the LA-PCR cloning technique, a gene homologous to *E. coli fepB* could be detected immediately upstream of *fepDGC*. The *E. coli* FepB represents a periplasmic binding protein involved in enterochelin uptake. The second transcriptional unit consists of a single gene that has high homology with the *E. coli fes* gene. It encodes an esterase which is involved in the release of iron from ferric enterochelin. The third transcriptional unit consists of two overlapping ORFs homologous to *fepA* of *E. coli*, indicating that a frameshift mutation might have taken place in *fepA* of *Y. enterocolitica* O8. In line with this observation, T7 polymerase expression of the third transcriptional unit leads to a truncated polypeptide of 35 kDa instead of about 83 kDa. However, a nonfunctional FepA protein does not exclude ferricatecholate siderophore uptake as has been shown for *E. coli* and *S. enterica*. For these bacteria, it is known that besides FepA other outer membrane receptor proteins such as Fiu, Cir, and IroN can support catecholate siderophore transport through the outer membrane (4, 36). The catecholate-like receptor CccA of *Y. enterocolitica* O8 (5) and two further iron-repressible outer membrane proteins of 75 and 90 kDa which might be involved in ferric enterochelin uptake have been described (45). In spite of this ambiguity concerning ferricatecholate siderophore transport through the outer membrane, we have shown that both *Yersinia fepDGC* and *fes* are functional in corresponding *E. coli* mutants. Moreover, inactivation of *fepDGC* or *fes* in *Y. enterocolitica* results in abrogation of ferric enterochelin uptake. In summary, *Y. enterocolitica* is endowed with the required genes for ferric enterochelin uptake (*fepDGC* and *fepB*) and utilization (*fes*).

The high degree of amino acid sequence similarity between *Y. enterocolitica* and *E. coli* Fes as well as between FepDGC and FepB indicates a common evolutionary origin (Fig. 7). In addition, the overall G+C content of the *Yersinia fes-fep* gene cluster (49%) is close to the average G+C content of *Y. enterocolitica* (46 to 48%) and lower than that of the *E. coli fes-fep* gene cluster (54%). Taken together, these data are suggestive of an early divergent evolution of the *fes-fep* gene cluster within members of the family *Enterobacteriaceae*. However, comparison of the gene arrangement of the *fes-fep* gene cluster of *E. coli* with that of *Y. enterocolitica* reveals some striking differences (Fig. 2). The *entF* and *fepE* homologues of *E. coli* are missing within the 7.5-kb *fes-fep* gene cluster of *Yersinia*.

To explain the absence of *fepE* in the *fes-fep* gene cluster of *Y. enterocolitica*, one has to consider the function of the *fepE* product in *E. coli*. FepE of *E. coli* had originally been thought to be involved in ferric enterochelin uptake. However, a recent study has demonstrated high sequence identity between *fepE* of *E. coli* and the *cld* gene of *Shigella flexneri* encoding the lipopolysaccharide chain length determinant Cld (26). Moreover, the G+C content of *fepE* (46%) is distinct from that of other *fep* genes in *E. coli* (55 to 61%), suggesting different origins. Thus, *fepE* might not be involved in ferric enterochelin uptake. It is therefore not surprising that *Y. enterocolitica* can utilize ferric enterochelin in spite of the absence of *fepE* in the *fes-fep* gene cluster. Beside *entF* and *fepE*, the *entD* gene is also missing in the enterochelin gene cluster of *Yersinia*. These genes might have been deleted because they are not necessary or of no advantage for *Y. enterocolitica*. On the other hand, it is unknown whether the *E. coli* enterochelin gene cluster (including the *fes*, *fep*, and *ent* genes) might have evolved by stepwise accumulation of coherent functional clusters (e.g., biosynthesis module and transport module) with subsequent rearrangements resulting in the functional mixed enterochelin

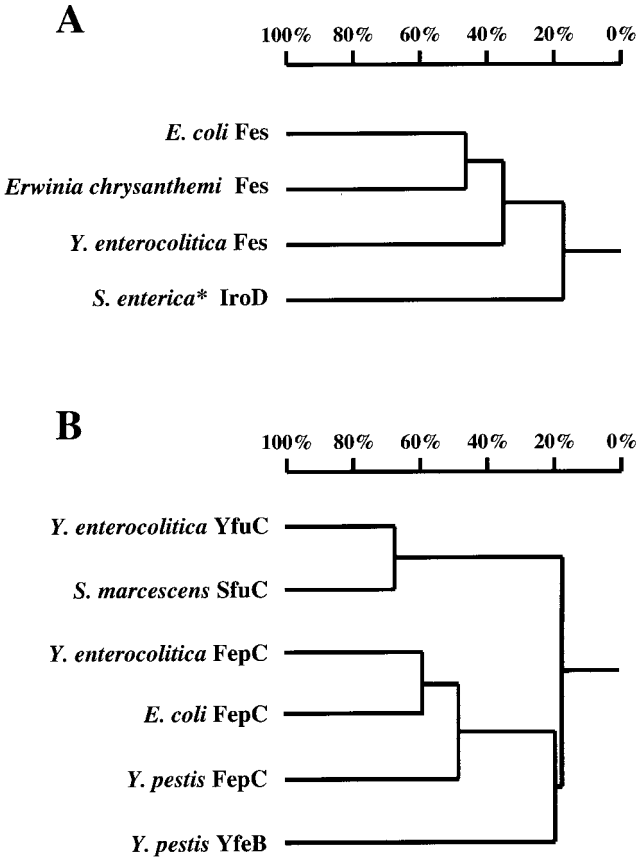

FIG. 7. Homology tree determined by pairwise alignment of amino acid sequences by the method developed by Wilbur and Lipman (1, 60). (A) Homology tree of *E. coli* Fes, *E. chrysanthemi* Fes, *Y. enterocolitica* Fes, and IroD of *S. enterica* (*, *Salmonella enterica* subsp. *enterica* serotype Typhi). (B) Homology tree for iron transport proteins belonging to the superfamily of ABC transporters: *Y. enterocolitica* YfuC, *S. marcescens* SfuC (2), *Y. enterocolitica* FepC, *E. coli* FepC, *Y. pestis* FepC, and *Y. pestis* YfeB (8).

cluster organization. Thus, as an alternative to the hypothesis of *ent-fepE* deletion in *Yersinia*, it is also conceivable that the *fepA-fes-fepDGC* cluster (including *fepB* upstream of *fepDGC*) is the conserved descendant of an ancestor of a catecholate siderophore transport or utilization module, with an insertion of *entF* and *fepE* in the *E. coli* enterochelin gene cluster at a later evolutionary stage. This question may be answered after analyzing the enterochelin gene cluster of other members of the family *Enterobacteriaceae*.

Furthermore, in this study we investigated the presence of the *fes* gene in pathogenic species of *Yersinia* by Southern hybridization and DNA sequencing. Surprisingly, the *Yersinia fes* probe hybridized exclusively with genomic DNA of different serotypes and biogroups of *Y. enterocolitica*. However, the *fes-fep* gene cluster was not detectable in *Y. enterocolitica* serotype O3 biogroup 4, *Y. pestis*, and *Y. pseudotuberculosis* as well as nonpathogenic *Yersinia* spp. (*Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii*). The *fes* gene sequences of different serotypes showed high identity (98.6 to 100%), indicating a common origin. The distribution of the *fes-fep* gene cluster is in good agreement with the results of the enterochelin feeding bioassay obtained for the corresponding strains.

Other iron uptake systems are known for *Yersinia* species. For *Y. enterocolitica*, the *yfu* gene (46) which has high similarity to the Sfu iron uptake system of *Serratia marcescens* has been described (40). Recently, an ABC transporter system for iron uptake (*yfe* cluster) in *Y. pestis* (8) that is also present in *Y. pseudotuberculosis* and *Y. enterocolitica* has been discovered. This Yfe system restored growth of an *E. coli* mutant deficient in enterochelin biosynthesis. However, it remains to be clarified how far the Yfe system is involved in ferric enterochelin uptake, since the Yfe system is involved in transport of other metal ions in addition to iron (7, 8). Moreover, putative proteins with similarity to *E. coli* FepDGC are deposited in the *Y. pestis* genome database (61), whereas no Fes-homologous polypeptide is present. In *Y. pestis*, the *fepDGC*-homologous genes are arranged in a single operon consisting of six ORFs. The deduced amino acid sequence of one ORF reveals similarity to the ferripyoverdin receptor and to the *E. coli* ferrioxamine B-ferricoprogen receptor but has only moderate similarity to the corresponding FepA protein of *Y. enterocolitica* described in this study. Of note, in *Y. pestis* as in *Y. enterocolitica*, no *ent*-orthologous genes encoding enterochelin biosynthesis enzymes are present in the neighborhood of the *fepDGC*-homologous gene locus. Figure 7 shows the homology trees determined by pairwise alignment of amino acid sequences of the different ferric enterochelin esterase orthologs (Fig. 7A) and the different ABC iron uptake transporter proteins identified in *Yersinia* species and other members of the family *Enterobacteriaceae* as well as in *E. chrysanthemi* (Fig. 7B) (1, 60).

In summary, the results of this study are in line with the observation that *Y. enterocolitica* can utilize catecholate siderophores but is unable to produce enterochelin. These results also emphasize the role of *fes*- and *fepDGC*-orthologous genes for active transport across the cytoplasmic membrane as well as for utilization of diverse catecholate siderophores. Catecholate siderophore receptors in the outer membrane are frequently targets of bacteriocins or phages: for example, the enterochelin receptor FepA is a target for the bacteriocin colicin B. In the case of *Yersinia*, colicin B-producing members of the *Enterobacteriaceae* may have selected for FepA-negative *Y. enterocolitica* strains without abolishing ferric enterochelin uptake. Thus, the *fep-fes* gene cluster may contribute to better survival of yersiniae in ecological niches such as the gut lumen and the environment. Studies are under way to clarify this hypothesis.

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