Genetic and Functional Properties of the Self-Transmissible *Yersinia enterocolitica* Plasmid pYE854, Which Mobilizes the Virulence Plasmid pYV[†]

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***Yersinia* strains frequently harbor plasmids, of which the virulence plasmid pYV, indigenous in pathogenic strains, has been thoroughly characterized during the last decades. Yet, it has been unknown whether the nonconjugative pYV can be transferred by helper plasmids naturally occurring in this genus. We have isolated the conjugative plasmids pYE854 (95.5 kb) and pYE966 (70 kb) from a nonpathogenic and a pathogenic *Yersinia enterocolitica* strain, respectively, and demonstrate that both plasmids are able to mobilize pYV. The complete sequence of pYE854 has been determined. The transfer proteins and *oriT* of the plasmid reveal similarities to the F factor. However, the pYE854 replicon does not belong to the IncF group and is more closely related to a plasmid of gram-positive bacteria. Plasmid pYE966 is very similar to pYE854 but lacks two DNA regions of the larger plasmid that are dispensable for conjugation.**

There is only scarce information available about self-transmissible plasmids in *Yersinia*. All pathogenic *Yersinia* strains harbor a conserved nonconjugative 70-kb virulence plasmid (pYV) encoding a type III secretion/transfer system and Yops (*Yersinia* outer proteins), which play a major role in virulence (10). Loss of the plasmid leads to a loss of virulence (42). Since the plasmid contains a gene for a lipoprotein that is 80% identical with the TraT proteins of F and R100, it has been suggested that pYV at one time was a conjugative plasmid (1, 7). Moreover, pYV of *Yersinia pestis* can be mobilized in *Escherichia coli* by the F factor, indicating that it contains a functional transfer origin (*oriT*). Allen et al. (1) identified a recombinant plasmid containing a 5.5-kb fragment of pYV which was efficiently mobilized by F. The restriction fragment was mapped and assigned to be part of the pYV low-calcium response (*lcr*) region. Yet the *oriT* sequence of this plasmid has not been determined. The *lcr* region of the *Yersinia* virulence plasmid is conserved in the three medically important species, which implies that the pYV plasmids of all pathogenic *Yersinia* strains are probably mobilizable. As F is not common in *Yersinia*, the question arises whether endogenous *Yersinia* plasmids are also able to mobilize pYV. Cornelis et al. (9) reported on a conjugative *lac* plasmid that is freely transmissible between strains of *Yersinia enterocolitica* and *E. coli*. Other authors described self-transmissible R plasmids in *Yersinia* that confer resistance against various antibiotics (20, 23, 24, 35). The conjugation of a streptomycin resistance plasmid between *E. coli* and *Y. pestis* was even demonstrated in the flea midgut (32). Strauch et al. (48) analyzed a recombinant cosmid con-

taining the conjugative transfer system of a cryptic *Y. enterocolitica* plasmid. However, none of these plasmids was investigated in terms of its potential to mobilize pYV. Furthermore, sequence data disclosing the relationship of the *Yersinia* plasmids to other plasmids are missing. This lack of knowledge prompted us to screen our *Yersinia* strain collection for the presence of large plasmids that were marked by antibiotic resistance genes and to use them for mating experiments. Two related self-transmissible plasmids have been isolated: pYE854 (95.5 kb) from the nonpathogenic *Y. enterocolitica* serogroup O:5 biogroup 1A strain 29854 and pYE966 (approximately 70 kb) from the pathogenic *Y. enterocolitica* serogroup O:5,27 biogroup 2 strain 966/89. Basic characteristics of the plasmids have been previously described (29).

Here, we present the complete sequence analysis of plasmid pYE854 and data on the conjugative properties of the plasmid, including its capability to mobilize the *Yersinia* virulence plasmid pYV. The study was undertaken to assess the potential for pYV dissemination in *Yersinia* populations. *Y. enterocolitica* is a heterogeneous species consisting of approximately 70 serogroups, of which only some (O:3, O:8, O:9, O:5,27) are known to be pathogenic in humans (4). Reports describing pYV in environmental *Yersinia* strains are yet not available, but some strains harbor plasmids displaying homologies to pYV (33). We therefore wanted to find out whether gene exchange by conjugation might be common between *Yersinia* strains.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The strains used in this study are listed in Table 1. *Escherichia coli* strains DH5 α (51) and GeneHogs (Invitrogen) were used as hosts for cloning procedures. All strains were grown in Luria-Bertani (LB) broth. Apart from *Yersinia* strains, which were cultivated at 28°C, all strains were incubated at 37°C. Solid agar media contained 1.8% (wt/vol) agar. When required, ampicillin and kanamycin were supplemented at 100 μ g ml⁻¹ and chloramphenicol and tetracycline at 12.5 μ g ml⁻¹.

Conjugation and mobilization experiments. Matings in which *Yersinia* strains were used as donor or recipient were carried out at 28°C. All other mating experiments were conducted at 37°C. Strains were grown in LB broth to an A₆₀₀

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Source/description	Reference or source
<i>E. coli</i> strains		
DH5 α	F ⁻ ϕ 80 Δ lacZ Δ M15 Δ (lacZYAargF)U169 <i>deoR recA1 endA1 hsdR17</i> (r _K ⁻ m _K ⁺) <i>phoA supE44</i> λ ⁻ <i>thi1 gyrA96 relA1</i>	51
S17-1 λ pir	λ pir (<i>recA thi pro hsdR</i> [res ⁻ mod ⁺] [RP4::2-Tc ^r ::Mu-Km ^r ::Tn7] λ pir phage lysogen)	47
Genehogs	F ⁻ <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) ϕ 80lacZ Δ M15 Δ lacX74 <i>recA1 araD139</i> Δ (<i>ara-leu</i>)7697 <i>galU galK rpsL</i> (Sm ^r) <i>endA1 nupG fhuA</i> ::IS2 (confers phage T1 resistance)	Invitrogen
<i>Y. enterocolitica</i> strains		
13169	Serogroup O:3; biogroup 4; recovered from pig	38, 39
29854	Serogroup O:5; biogroup 1A; recovered from food	29, 30, 39
83/88	Serogroup O:5,27; biogroup 3; recovered from food	30, 31
966/89	Serogroup O:5,27; biogroup 2; recovered from human	29
31080	Serogroup O:9; biogroup 2; recovered from pig	This work
12	Serogroup O:13,7; biogroup 1A; recovered from water	This work
<i>Y. pseudotuberculosis</i> 1340	Serogroup I; recovered from animal	P. Dersch
Other strains		
<i>Citrobacter freundii</i> CB1614	Reference strain BfR	49
<i>Escherichia hermannii</i> 4560	Reference strain BfR	This work
<i>Salmonella enterica</i> serovar Typhimurium type I	Reference strain BfR	49
<i>Shigella flexneri</i> 6:88 Boyd	Reference strain BfR	This work
Plasmids		
pYE854	95 kb; isolated from <i>Y. enterocolitica</i> strain 29854	This work
pYE854 11/01	pYE854 (Km ^r insertion at position 47050)	This work
pYE966	70 kb; isolated from <i>Y. enterocolitica</i> strain 966/89	This work
p1340	pYV (Cm ^r) of <i>Y. pseudotuberculosis</i> strain 1340	P. Dersch
pBR327	3.3-kb derivative of pBR322; Ap ^r Tc ^r	12
pBR329	4.2-kb derivative of pBR322; Ap ^r Tc ^r Cm ^r	11
pIV2	<i>Yersinia</i> cloning vector; Km ^r	49
pLitmus38	Cloning vector; Ap ^r	New England Biolabs
pUC19	Cloning vector; Ap ^r	New England Biolabs
pUTKm	7.1-kb R6K-derived suicide vector; Ap ^r Km ^r	13, 28
pUB110	4.5-kb <i>Bacillus/Staphylococcus</i> vector; Km ^r	36
mini-Tn5 Cm	R6K-derived suicide vector; Cm ^r	13
pJH808	pIV2 ClaI Ω (pYE854 [3,927 bp, ClaI])	This work
pJH907	pIV2 HindIII Ω (pYE854 [4,264 bp, HindIII])	This work
pJH907-A	pIV2 Δ (HindIII-EcoRI) Ω (pYE854 [2,404 bp, HindIII-EcoRI])	This work
pJH907-B	pIV2 Δ (HindIII-EcoRI) Ω (pYE854 [1,860 bp, HindIII-EcoRI])	This work
pJH907-C	pIV2 Δ (HindIII-EcoRI) Ω (pYE854 [1,016 bp, HindIII-EcoRI])	This work
pJH907-D	pIV2 Δ (HindIII-EcoRI) Ω (pYE854 [666 bp, HindIII-EcoRI])	This work
pJH907-E	pIV2 Δ (HindIII-EcoRI) Ω (pYE854 [659 bp, HindIII-EcoRI])	This work
pJH907-F	pIV2 Δ (HindIII-EcoRI) Ω (pYE854 [309 bp, HindIII-EcoRI])	This work
pJH907-G	pIV2 Δ (HindIII-EcoRI) Ω (pYE854 [272 bp, HindIII-EcoRI])	This work
pJH1010	pYE854 PstI (41056–91245::Tc ^r)	This work
pJH1011	pYE854 BsePI (68129–95275::Tc ^r)	This work
pJH1012	pYE854 PmeI/SmaI (75368–94503::Tc ^r)	This work
pJH1013	pYE854 PmeI/StuI (75368–86673::Tc ^r)	This work
pJH1014	pYE854 EcoRI (75988–85351::Tc ^r)	This work
pJH1015	pYE854 NdeI (76227–84577::Tc ^r)	This work

of 0.3. A 500- μ l amount of the donor bacteria was mixed with 4.5 ml of the recipient. The bacteria were sedimented at 5,000 $\times$ g for 5 min, resuspended in 100 μ l LB broth, and streaked on a Millipore filter (pore size, 0.45 μ m; diameter, 25 mm) placed on an LB agar plate (8). The filters were incubated for 12 h at 28°C or 37°C. Thereafter, cells were suspended in 1 ml 0.7% saline solution and 100- μ l aliquots of serial dilutions were plated on selective agar containing the appropriate antibiotics. Transfer frequencies were calculated by the ratio of transconjugants per donor cell.

In vivo mutagenesis of the conjugative plasmids. Plasmids containing either a kanamycin (Km^r) or a chloramphenicol acetyltransferase (Cm^r) resistance gene were created by in vivo transposon mutagenesis of the host strains using the mini-Tn5 transposon derivative pUT described by de Lorenzo et al. (13) and Herrero et al. (28). *Y. enterocolitica* strains harboring plasmid pYE854 or pYE966 were used as recipients for filter mating experiments with *E. coli* strain S17-1 λ pir containing pUTKm or mini-Tn5 Cm (Table 1). Transconjugants were selected on CIN agar (45) supplemented with the appropriate antibiotic. To

TABLE 2. Characterization of pYE854 mutants

Mutant	Position/ insertion	ORF(s) ^a	Related protein(s) and source	Conjugation/mobilization ^d of pYV ¹³⁴⁰
A1/01	1157	001	Transposase (<i>Y. pestis</i> KIM)	+
A1/02	3261	—		+
A1/03	3872	007	None found	+
P2/01	6288	011 ^c	Hypothetical protein (<i>Y. bercovieri</i> ATCC 43970)	+
P2/02	6413	011 ^c	Hypothetical protein (<i>Y. bercovieri</i> ATCC 43970)	+
M2/01	13644	025	None found	+
M2/02	19235	036	TraI; conjugative transfer relaxase/helicase (<i>S. flexneri</i> plasmid R100)	—
M2/03	19404	036	TraI; conjugative transfer relaxase/helicase (<i>S. flexneri</i> plasmid R100)	—
S1/01	18341	036 ^c	TraI; conjugative transfer relaxase/helicase (<i>S. flexneri</i> plasmid R100)	—
S1/02	18996	036	TraI; conjugative transfer relaxase/helicase (<i>S. flexneri</i> plasmid R100)	—
E1/01	21567	036 ^c	TraI; conjugative transfer relaxase/helicase (<i>S. flexneri</i> plasmid R100)	—
E1/02	20783	036	TraI; conjugative transfer relaxase/helicase (<i>S. flexneri</i> plasmid R100)	—
E1/03	20030	036	TraI; conjugative transfer relaxase/helicase (<i>S. flexneri</i> plasmid R100)	—
B1/01	40209	094	Transposase (<i>Y. pestis</i> strain 91001)	+
B1/02	43850	100	Transposase (<i>E. coli</i> plasmid F)	+
B1/03	44301	100 ^c	Transposase (<i>E. coli</i> plasmid F)	+
I1/01	47050	—		+
K3/01	49860	121	Hypothetical protein (<i>Y. pseudotuberculosis</i> IP 31758)	+
T1/01	50299	122	Permease (<i>Y. bercovieri</i> ATCC 43970)	—
T1/02	50685	122	Permease (<i>Y. bercovieri</i> ATCC 43970)	—
R1/01	52439	127	Hypothetical protein (<i>Magnetospirillum magneticum</i> AMB-1)	+
G2/01	55264	142 ^c	DNA transfer and F pilus assembly protein (<i>Sphingomonas</i> sp. SKA58)	—
D1/01	59112	144	TraH; conjugative transfer protein precursor (<i>S. flexneri</i> plasmid R100)	—
D1/02	60919	147	Putative lipoprotein (<i>Porphyromonas gingivalis</i> W83)	—
O1/01	63138	156 ^c	TraU; conjugative transfer protein precursor (<i>S. flexneri</i> plasmid R100)	—
H1/01	65749	162	TraZ; conjugative transfer protein (<i>S. flexneri</i> plasmid R100)	—
H1/02	66001	164	TraN; type IV conjugative transfer system protein (<i>Yersinia ruckeri</i>)	+ ^b
H1/03	66255	164	TraN; type IV conjugative transfer system protein (<i>Y. ruckeri</i>)	+ ^b
G1/01	72004	171	TraB; conjugative transfer protein (<i>E. coli</i> plasmid F)	—
R2/01	76626	183	GpA; phage terminase (<i>Shewanella denitrificans</i> OS217)	+
C1/01	84818	203	None found	+
C1/02	86989	209	Transposase (<i>Y. pestis</i> strain 91001)	+
C1/03	88157	211; 212	Transposase (<i>Y. bercovieri</i> ATCC 43970); prophage integrase (partial) (<i>Erwinia carotovora</i> sp. <i>atroseptica</i> SCR11043)	+
P1/01	90939	219 ^c	Cation efflux protein (<i>Dechloromonas aromatica</i> RCB)	+
A1/04	93076	224 ^c	Transposase (<i>Proteus vulgaris</i>)	+

^a A dash indicates that insertion occurred within an intergenic sequence.

^b Transferred with reduced frequencies.

^c Several ORFs are affected. See Table S2 in the supplemental material.

^d +, conjugation/mobilization occurred; —, conjugation/mobilization did not occur.

detect plasmids in which the resistance gene had been inserted, plasmid DNA was isolated from the mutants and subjected to restriction analysis and hybridization with the transposon delivery vectors. The insertion sites of the resistance genes were determined by cloning DraI restriction fragments of the pYE854 mutants with the help of the vector pLitmus38 (Ap^r; New England Biolabs). Sequencing was performed using primers deduced from the marker genes.

Nucleotide sequence determination and analysis of plasmid pYE854. For shotgun sequencing of pYE854, a fragment library was constructed. Small (1.8 to 2.2-kb) DNA fragments of the plasmid were generated by ultrasonic treatment. After end repair with T4 polymerase (Roche), 10 µg DNA was loaded onto an agarose gel (0.9%). DNA fragments of the appropriate size were excised from the gel. The extracted DNA was cloned into the vector pUC19 cleaved with SmaI (Roche).

DNA sequencing reactions were set up using an Applied Biosystems BigDye Terminator version 3.1 cycle sequencing kit. We have sequenced the shotgun clones from both sides up to a sixfold genome coverage using an ABI 3730 XL sequencer. The data assembly was accomplished by using Staden Package software version 4.6 (Roger Staden, Cambridge, United Kingdom). Gap closure was performed by combinatorial PCR and sequencing of the generated PCR fragments. Sequence analyses were performed by using the MacVector 8.0 software of the Oxford Molecular Group. Blast searches were carried out with the NCBI database, and the GAP program of the GCG package (14) was used for calculating similarity and identity values.

To determine putative promoters, the 300-bp upstream regions of pYE854 open reading frames (ORFs) were analyzed for the existence of –35 and –10 consensus sequences (TTGACA-N_{15–20}-TATAAT) described by Hawley and McClure (27) and Harley and Reynolds (26). To identify the origin of transfer (*oriT*) of pYE854, the complete nucleotide sequence was analyzed for the existence of consensus sequences for the *oriT* *nic* region families described by Zechner et al. (52).

Construction of replicative miniplasmid derivatives of pYE854. For all DNA manipulations, standard techniques were applied (44). To identify regions essential for plasmid replication, miniplasmid derivatives of pYE854 were created by cleavage of the plasmid DNA with various restriction endonucleases. Restriction fragments bearing overhanging ends were trimmed with T4 polymerase (New England Biolabs). The tetracycline resistance gene (*tetA*) of Tn5 was amplified by PCR using plasmid pBR327 as template and was ligated to the restriction fragments of pYE854.

Cloning of restriction fragments and of the *oriT* region of plasmid pYE854. Covalently closed circular plasmid DNA purified by cesium chloride gradient centrifugation (44) was digested with the restriction endonucleases ClaI and HindIII. The fragments were inserted into the corresponding sites of pIV2 (Km^r) (49). The constructs were introduced into the *E. coli* strain GeneHogs. Recombinant plasmids were characterized by restriction analysis and sequencing. Plasmids containing ClaI restriction fragments resulted in the pJH800 series, and those harboring HindIII restriction fragments gave the pJH900 series (see Fig. 4).

TABLE 3. Results of the mating experiments with various recipient strains

Recipient species, serogroup, or type	No. of strains tested	No. of strains in which transfer of pYE854 occurred ^a
<i>Y. enterocolitica</i> serogroups	25	24/25
O:3	7	7/7
O:5	5	4/5
O:5,27	4	4/4
O:7,8	1	1/1
O:8	4	4/4
O:9	4	4/4
Other <i>Yersinia</i> spp.		
<i>Y. pseudotuberculosis</i>	2	2/2
<i>Y. intermedia</i>	2	2/2
<i>Y. frederiksenii</i>	2	2/2
<i>Y. kristensenii</i>	2	2/2
<i>Y. mollaretii</i>	2	1/2
<i>Agrobacterium tumefaciens</i>	1	0/1
<i>Acinetobacter calcoaceticus</i>	1	0/1
<i>Bacillus</i> spp.	8	0/8
<i>B. subtilis</i>	2	0/2
<i>B. licheniformis</i>	2	0/2
<i>B. megaterium</i>	2	0/2
<i>B. thuringiensis</i>	2	0/2
<i>Citrobacter freundii</i>	1	1/1
<i>Escherichia</i> spp.	5	5/5
<i>E. coli</i>	4	4/4
<i>E. hermannii</i>	1	1/1
<i>Enterobacter cloacae</i>	1	0/1
<i>Proteus mirabilis</i>	1	0/1
<i>Pseudomonas</i> spp.	2	0/2
<i>P. aeruginosa</i>	1	0/1
<i>P. putida</i>	1	0/1
<i>Salmonella enterica</i> serovar Typhimurium	2	2/2
<i>S. enterica</i> serovar Typhimurium type I	1	1/1
<i>S. enterica</i> serovar Typhimurium type III	1	1/1
<i>Serratia marcescens</i>	1	0/1
<i>Shigella flexneri</i>	1	1/1

^a The donor strain for all mating experiments was *Y. enterocolitica* 83/88 (O:5,27).

One of the recombinant plasmids described above, pJH907, was shown to be mobilizable by plasmid pYE854. To identify the *oriT* sequence on pJH907, its 4.2-kb HindIII insert was digested with EcoRI, yielding two restriction fragments that were cloned by use of the vector pIV2, resulting in the plasmids pJH907-A and pJH907-B. *oriT* located on plasmid pJH907-B was defined by constructing smaller PCR fragments using forward primers containing a HindIII site and reverse primers containing an EcoRI site, respectively (see Table S1 in the supplemental material). The PCR products were digested with the corresponding restriction endonucleases and inserted into the EcoRI and HindIII sites of pIV2. *E. coli* DH5 α transformants containing one of the resulting plasmids, pJH907-C to -G, and either pYE854 or pYE966 were investigated in terms of mobilization of the constructs by the conjugative plasmids (see below).

Nucleotide sequence accession number. The complete sequence of plasmid pYE854 has been submitted to the EMBL databank under the accession number AM905950.

RESULTS

The self-transmissible plasmid pYE854 is able to mobilize pYV. Plasmid pYE854 was detected in nonpathogenic *Y. enterocolitica* biogroup 1A strain 29854 recovered from food (Table 1). Strain 29854 also contains the 46.3-kb linear plasmid prophage PY54 which has already been described in more detail (30, 31). To allow selection for pYE854, its host strain was subjected to in vivo mutagenesis with a kanamycin resistance gene (see Materials and Methods). Mutants containing the marker gene on pYE854 were detected by DNA hybridization and analyzed to determine the insertion site (Table 2). In addition, the conjugative properties of the mutants were studied. For filter mating experiments, mutant I1/01 was chosen as the donor in which the resistance gene had inserted within an intergenic region (plasmid position 47050), retaining high-frequency transmission of the plasmid. Selection of the recipients (Table 1) was achieved by transforming gram-negative strains with the vector pBR327 (Ap^r Tc^r) that is known to be nonmobilizable (12). The *Bacillus* strains possessed an intrinsic tetracycline resistance. Transconjugants were analyzed with respect to their plasmid content and by typing. We observed the transfer of pYE854-I1/01 into a wide range of *Yersinia* strains and into some other genera belonging to the family *Enterobacteriaceae*, e.g., *Citrobacter*, *Escherichia*, *Salmonella*, and *Shigella*, but not into *Bacillus* species (Table 3). The highest transfer frequencies (10^{-1} to 10^{-2} /donor) were obtained when *Yersinia* strains were used as donor and recipient, while the efficiencies of conjugation into other genera were determined to be several orders of magnitude lower (Table 4). We studied the stability of pYE854-I1/01 in *E. coli* for approx-

TABLE 4. Efficiencies of pYE854 and pYE966 conjugation and pYV mobilization

Donor (serogroup)	Recipient (serogroup)	Conjugative plasmid	Transfer rate	Mobilization of pYV ^{1340a}
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>Y. enterocolitica</i> 12 (O:13,7)	pYE854	1.2×10^{-1}	1.2×10^{-2}
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>Y. enterocolitica</i> 12 (O:13,7)	pYE966	9.4×10^0	9.6×10^{-1}
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>E. coli</i> K12 DH5 α	pYE854	6.3×10^{-5}	2.3×10^{-7}
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>E. coli</i> K12 DH5 α	pYE966	3.2×10^{-5}	9.7×10^{-6}
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>S. flexneri</i> 6:88 Boyd	pYE854	2.3×10^{-7}	7.4×10^{-8}
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>Escherichia hermannii</i> 4560	pYE854	7.7×10^{-7}	—
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>Citrobacter freundii</i> CB1614	pYE854	1.6×10^{-8}	—
<i>Y. enterocolitica</i> 83/88 (O:5,27)	<i>Salmonella enterica</i> serovar Typhimurium type I	pYE854	9.5×10^{-7}	—
<i>Y. pseudotuberculosis</i> (I)	<i>Y. enterocolitica</i> 83/88 (O:5,27)	pYE854	1.1×10^{-2}	1.1×10^{-5}
<i>Y. pseudotuberculosis</i> (I)	<i>Y. enterocolitica</i> 12 (O:13,7)	pYE854	1.1×10^{-6}	1.7×10^{-8}

^a A dash indicates that no mobilization was detectable.

FIG. 1. Plasmid analysis of donor strains, recipients, and transconjugants. All donor and recipient strains contained the plasmids pYE854/pYV and pBR327, respectively. (A) An 0.8% agarose gel showing DraI restriction patterns of the plasmids. Lane M, marker (λ Eco130I); lane 1, pYE854; lane 2, pYV (p1340); lane 3, *Y. pseudotuberculosis* 1340 (donor); lane 4, *E. coli* DH5 α (donor); lane 5, *Y. enterocolitica* 83/88 (recipient); lane 6, *Y. enterocolitica* 83/88 (transconjugant, donor strain was *Y. pseudotuberculosis* 1340); lane 7, *Y. enterocolitica* 83/88 (transconjugant, donor strain was *E. coli* DH5 α); lane 8, *Y. enterocolitica* 31080 (recipient); lane 9, *Y. enterocolitica* 31080 (transconjugant, donor strain was *Y. pseudotuberculosis* 1340); lane 10, *Y. enterocolitica* 31080 (transconjugant, donor strain was *E. coli* DH5 α); lane 11, *Y. enterocolitica* 12 (recipient); lane 12, *Y. enterocolitica* 12 (transconjugant, donor strain was *Y. pseudotuberculosis* 1340). (B) Southern hybridization of the plasmids shown in panel A to pYE854. Transconjugants obtained by using *E. coli* as donor were selected on agar containing chloramphenicol (pYV) and tetracycline (pBR327) and did not harbor pYE854 (lanes 7 and 10). (C) Southern hybridization of the plasmids shown in panel A to pYV (p1340). Arrows indicate restriction fragments that are different in the virulence plasmids of the recipients and the transconjugants.

imately 100 generations under nonselective conditions and could not detect any significant loss of the plasmid. Thus, pYE854 replication is not restricted to *Yersinia*. Furthermore, plasmid analyses of the transconjugants disclosed that the conjugative plasmid is compatible with the *Yersinia* virulence plasmid pYV (Fig. 1).

Up to now, it has not been elucidated whether pYV can be mobilized with the help of another plasmid naturally occurring

in this genus. To answer this question, plasmid pYE854-I1/01 was introduced into *Yersinia pseudotuberculosis* strain 1340 (Table 1), whose virulence plasmid (p1340) is marked with a *cat* gene inserted adjacent to *yadA*. Following this step, the resulting strain containing both plasmids was used as a donor strain in mating experiments with various recipients harboring pBR327. Triply resistant transconjugants containing the plasmids pYE854-I1/01, p1340, and pBR327 could be isolated

TABLE 5. Selected ORFs of plasmid pYE854

ORF	Start position	Stop position	Length (amino acids)	Molmass (kDa)	pI	Predicted function	Related protein(s) and source/description	E value	Extent of best match % identity/length of region (no. of amino acids)	NCBI accession no.
001	366	1388	340	37.83	9.97	Transposase	Transposase (<i>Y. pestis</i> KIM)	0.0	95/340	AAC82723.1
003	1803	1489	104	11.63	9.80	Unknown	Hypothetical protein YPA_MT0073 (<i>Y. pestis</i> Antiqua)	3.0E-06	84/26	YP_636761.1
004	1926	2243	105	12.10	9.90	Unknown	Hypothetical protein YR71pYRI_0023 (<i>Yersinia ruckeri</i>)	3.0E-15	47/97	YP_001101717.1
006	3096	2431	221	26.04	7.13	Unknown	Hypothetical protein AmetDRAFT_2972 (<i>Alkaliphilus metalliredigenes</i> QYMF)	2.0E-31	37/213	ZP_00799958.1
008	4048	5493	481	54.15	8.67	Methylase	Site-specific DNA methylase (<i>Y. pestis</i> biovar Orientalis strain IP275)	7.0E-116	45/470	ZP_01175891.1
010	5616	5993	125	14.16	7.13	Unknown	Hypothetical protein pCTX-M3_039 (<i>Citrobacter freundii</i>)	1.0E-15	39/112	NP_774998.1
011	6105	7598	497	56.27	7.95	Unknown	Hypothetical protein YberA_01003565 (<i>Y. bercovieri</i> ATCC 43970); putative DNA primase (<i>Clostridium difficile</i> 630)	2.0E-157; 5.0E-12	98/306; 23/394	ZP_00820298.1; YP_001087592.1
013	6562	6401	53	5.57	6.21	Unknown	Hypothetical protein Cpham1DRAFT_2669 (<i>Chlorobium phaeobacteroides</i> BS1)	1.2E-02	50/46	ZP_00531443.1
015	7761	8093	110	12.47	9.61	Addiction system (toxin)	Hypothetical protein Plu0205 (<i>Photobacterium luminescens</i> sp. <i>laumondii</i> TTO1); Gp49 (<i>E. coli</i> bacteriophage N15); Gp49 (<i>Klebsiella oxytoca</i> bacteriophage ϕ KO2)	1.0E-32; 2.0E-12; 6.0E-12	82/101; 40/103; 40/103	NP_927569.1; NP_046945.1; YP_006629.1
016	8086	8388	100	11.08	7.15	Addiction system (antitoxin)	Predicted transcriptional regulator (<i>Y. bercovieri</i> ATCC 43970); Gp48 (<i>E. coli</i> bacteriophage N15); Gp48 (<i>Klebsiella oxytoca</i> bacteriophage ϕ KO2)	4.0E-41; 1.0E-04; 2.0E-03	95/100; 32/83; 29/87	ZP_00820297.1; NP_046944.1; YP_006628.1
018	8863	9264	133	15.30	4.85	Dehydrogenase	Uncharacterized anaerobic dehydrogenase (<i>Y. bercovieri</i> ATCC 43970)	5.0E-46	89/110	ZP_00820229.1
019	9300	10109	269	31.24	5.08	Hydratase	Putative aconitate hydratase (<i>Geobacter lovleyi</i> SZ)	1.6E-02	37/67	ZP_01594594.1
021	10829	11230	133	15.30	4.85	Dehydrogenase	Uncharacterized anaerobic dehydrogenase (<i>Y. bercovieri</i> ATCC 43970)	5.0E-46	89/110	ZP_00820229.1
022	11266	12075	269	31.24	5.08	Hydratase	Putative aconitate hydratase (<i>Geobacter lovleyi</i> SZ)	1.6E-02	37/67	ZP_01594594.1

024	13055	13357	100	11.67	8.89	Unknown	Conserved hypothetical protein (<i>E. coli</i>)	1.0E-06	37/67	ABF67878.1
030	15280	17352	690	77.22	8.23	DNA transport	TrdA (<i>Legionella pneumophila</i> subsp. <i>pneumophila</i> strain Philadelphia 1); type IV secretory pathway, VirD4 component (<i>Vibrio vulnificus</i> CMCP6); coupling protein TraD (<i>E. coli</i> plasmid F)	2.0E-74; 5.0E-62; 8.0E-61	31/570; 27/597; 26/ 682	YP_096091.1; NP_762611.1; NP_061481.1
036	17353	22323	1656	186.80	8.47	<i>oriT</i> relaxase/helicase	Mobilization protein TraI (<i>E. coli</i>); TraI (<i>Salmonella enterica</i> serovar Typhimurium plasmid R46); conjugative transfer relaxase/helicase (<i>Shigella</i> <i>flexneri</i> plasmid R100)	1.0E-90; 2.0E-90; 4.0E-43	31/956; 31/956; 37/ 318	AF109305.3; NP_511201.1; NP_052981.1
040	22895	22368	175	19.72	9.83	Restriction endonuclease	Hypothetical protein pEL60p14 (<i>Erwinia amylovora</i>); hypothetical protein R46_003 (<i>Salmonella enterica</i> serovar Typhimurium plasmid R46); Restriction endonuclease (<i>Serratia proteamaculans</i> 568)	2.0E-32; 1.0E-31; 2.0E-30	49/139; 44/156; 45/ 140	NP_943213.1; NP_511181.1; ZP_01535230.1
043	24200	23568	210	23.40	9.62	Transglycosylase	Putative transglycosylase PilT (<i>Salmonella enterica</i> serovar Typhimurium plasmid R46); putative twitching motility protein PilT (<i>Sodalis</i> <i>glossinidius</i> strain <i>morsitans</i>); lytic transglycosylase (<i>E. coli</i> plasmid F)	2.0E-21; 5.0E-21; 3.0E-13	40/132; 42/163; 36/ 155	BAA77980.1; YP_456185.1; NP_061449.1
045	24758	25174	138	15.83	9.12	Transcription regulation	Histone-like nucleoid-structuring protein H-NS (<i>Shewanella</i> <i>putrefaciens</i> 200); DNA-binding protein H-NS (<i>Marinomonas</i> sp. MED121)	6.0E-04; 7.0E-03	27/118; 27/101	ZP_01704518.1; ZP_01076238.1
049	26280	25510	256	28.67	9.87	Integrase	Hypothetical protein Ypsel_02003938 (<i>Y. pseudotuberculosis</i> IP 31758); integrase COG0582 (<i>Y. bercovieri</i> ATCC 43970); site- specific recombinase Int (<i>E. coli</i> plasmid F)	3.0E-140; 2.0E-137; 1.0E-45	99/256; 97/256; 46/ 246	ZP_01494755.1; ZP_00821919.1; NP_061423.1
055	27837	27067	256	28.55	5.52	Transporter	Hypothetical protein AGR_pat_528 (<i>Agrobacterium tumefaciens</i> strain C58); autotransporter protein (<i>Agrobacterium tumefaciens</i> strain C58); type IV secretory pathway, adhesin AidA (<i>Burkholderia cenocepacia</i> PC184)	5.0E-21; 9.0E-21; 7.0E-15	27/262; 27/262; 27/ 251	NP_396297.1; NP_535736.1; ZP_00978381.1

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TABLE 5—Continued

ORF	Start position	Stop position	Length (amino acids)	Molmass (kDa)	pI	Predicted function	Related protein(s) and source/description	E value	Extent of best match % identity/length of region (no. of amino acids)	NCBI accession no.
059	29753	27822	643	66.20	4.60	Unknown	Filamentous hemagglutinin, adhesin HecA repeat ×2 (<i>Ralstonia eutrophia</i> JMP134); serine protease EspC precursor (enteropathogenic <i>E. coli</i> -secreted protein C)	3.0E-03; 4.0E-03	23/451; 28/240	YP_294319.1; ESPC_ECO27
062	32162	29763	799	87.93	8.73	Hyaluronate lyase	Hypothetical protein VV0534 (<i>Vibrio vulnificus</i> YJ016); hypothetical protein VV0533 (<i>Vibrio vulnificus</i> YJ016); hyaluronate lyase precursor (<i>Vibrio fischeri</i> ES114)	7.0E-146; 4.0E-123; 3.0E-116	39/813; 34/781; 34/769	NP_933327.1; NP_933326.1; YP_206952.1
066	33185	32181	334	39.39	9.05	Unknown	Hypothetical protein VV0535 (<i>Vibrio vulnificus</i> YJ016)	9.0E-38	33/339	NP_933328.1
070	34696	33230	488	56.39	7.51	Unknown	Hypothetical protein PCNPT3_11983 (<i>Psychromonas</i> sp. CNPT3); hypothetical protein VV0544 (<i>Vibrio vulnificus</i> YJ016)	4.0E-131; 3.0E-119	46/493; 44/492	ZP_01217030.1; NP_933337.1
073	35755	34721	344	39.97	5.21	Glucuronyl hydrolase	Hypothetical protein VV0541 (<i>Vibrio vulnificus</i> YJ016); hypothetical protein PCNPT3_11988 (<i>Psychromonas</i> sp. CNPT3); predicted unsaturated glucuronyl hydrolase (<i>Y. pestis</i> Angola)	2.0E-111; 6.0E-108; 5.0E-107	58/334; 53/341; 55/341	NP_933334.1; ZP_01217031.1; ZP_00796104.1
075	36272	35835	145	15.52	4.11	PTS	PTS, mannose/fructose-specific component IIA (<i>Vibrio vulnificus</i> YJ016); PTS, N-acetylglactosamine-specific IIA component (<i>Psychromonas</i> sp. CNPT3)	2.0E-34; 3.0E-26	58/124; 50/124	NP_933333.1; ZP_01217033.1
079	37151	36282	289	31.45	8.16	PTS	PTS, mannose/fructose/N-acetylglactosamine-specific component IID (<i>Vibrio vulnificus</i> YJ016); PTS, N-acetylglactosamine-specific IID component (<i>Psychromonas</i> sp. CNPT3)	9.0E-103; 2.0E-87	61/293; 57/292	NP_933332.1; ZP_01217034.1
082	37908	37138	256	27.41	4.90	PTS	PTS, mannose/fructose/N-acetylglactosamine-specific component IIC (<i>Vibrio vulnificus</i> YJ016); PTS, N-acetylglactosamine-specific IIC component (<i>Psychromonas</i> sp. CNPT3)	3.0E-82; 6.0E-58	63/254; 50/256	NP_933331.1; ZP_01217035.1

086	38401	37928	157	17.59	9.37	PTS	PTS, mannose/fructose/N-acetylgalactosamine-specific component IIB (<i>Vibrio vulnificus</i> YJ016); PTS, N-acetylgalactosamine-specific IIB component (<i>Psychromonas</i> sp. CNPT3)	1.0E-43; 3.0E-38	55/155; 51/158	NP_933330.1; ZP_01217036.1
089	39302	38514	262	29.27	5.51	Transcriptional regulator	Transcriptional regulator of sugar metabolism (<i>Vibrio vulnificus</i> YJ016); transcriptional repressor of <i>aga</i> operon (<i>Psychromonas</i> sp. CNPT3)	2.0E-29; 1.0E-11	33/246; 25/243	NP_933329.1; ZP_01217450.1
090	39492	39749	85	9.64	10.03	Unknown	Hypothetical protein YPA_MT0073 (<i>Y. pestis</i> Antiqua)	2.0E-06	84/26	YP_636761.1
094	40872	39850	340	37.82	9.97	Transposase	Probable transposase (<i>Y. pestis</i> biovar Microtus strain 91001)	0.0	94/340	NP_995565.1
097	41237	40896	113	12.57	7.02	ATPase	Hypothetical protein R64_p028 (<i>Salmonella enterica</i> serovar Typhimurium plasmid R64); oxyanion-translocating ATPase (<i>Y. intermedia</i> ATCC 29909); arsenical pump-driving ATPase (<i>Y. enterocolitica</i> 8081)	6.0E-20; 8.0E-15; 1.0E-14	97/48; 78/47; 55/70	NP_863383.1; ZP_00834873.1; YP_001007632.1
099	41902	41246	218	24.52	9.90	Recombinase	Recombinase (<i>Salmonella enterica</i> serovar Typhimurium plasmid R46); putative resolvase (<i>Y. pestis</i> biovar Microtus strain 91001)	7.0E-104; 1.0E-85	98/218; 81/209	NP_511241.1; NP_995411.1
100	41982	44990	1002	113.71	8.84	Transposase	Transposase (<i>E. coli</i> plasmid F)	0.0	91/1001	NP_061389.1
102	42122	41964	52	5.62	9.81	Unknown	Hypothetical protein (<i>E. coli</i>)	3.0E-02	53/39	ABG49234.1
111	45167	46048	293	33.97	6.54	Restriction endonuclease	ENSANGP0000029959 (<i>Anopheles gambiae</i> strain PEST); restriction endonuclease (<i>E. coli</i> F11)	2.0E-112; 8.0E-80	69/289; 52/281	XP_001230420.1; ZP_00726032.1
115	46977	46144	277	32.05	4.49	Unknown	W0044 (<i>E. coli</i>)	3.0E-19	29/257	AF401292_38
116	47513	48139	208	24.42	9.24	Unknown	Hypothetical protein XOO2824 (<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> KACC10331)	8.0E-08	25/151	YP_201463.1
121	49871	49107	254	29.92	5.14	Unknown	Hypothetical protein Ypsel_02003937 (<i>Y. pseudotuberculosis</i> IP 31758)	2.0E-20	35/223	ZP_01494754.1
122	50121	50852	243	27.43	8.95	Permease	Hypothetical protein YPTB1564 (<i>Y. pseudotuberculosis</i> IP 32953); permease of the major facilitator superfamily (<i>Y. bercovieri</i> ATCC 43970)	2.0E-63; 3.0E-06	56/214; 25/182	YP_070092.1; ZP_00821712.1
125	51098	51577	159	17.44	8.27	Unknown	Hemolysin-coregulated protein (<i>Burkholderia dolosa</i> AU0158)	3.0E-45	56/157	EAY70565.1

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TABLE 5—Continued

ORF	Start position	Stop position	Length (amino acids)	Molmass (kDa)	pI	Predicted function	Related protein(s) and source/description	E value	Extent of best match % identity/length of region (no. of amino acids)	NCBI accession no.
127	51850	52896	348	39.58	9.11	Unknown	Hypothetical protein Amb1131 (<i>Magnetospirillum magnetotum</i> AMB-1)	1.0E-16	31/238	YP_420494.1
129	53194	53445	83	9.48	7.15	Transcriptional regulator	Transcriptional regulator, XRE family (<i>Serratia proteamaculans</i> 568); putative DNA-binding protein (<i>Y. enterocolitica</i> 8081)	7.0E-28; 8.0E-24	71/82; 63/83	ZP_01535204.1; YP_001007657.1
142	58491	54838	1217	128.71	4.92	Pilus assembly	Exoprotein involved in heme utilization or adhesion (<i>Y. bercovieri</i> ATCC 43970); DNA transfer and F pilus assembly protein (<i>Sphingomonas</i> sp. SKA58); pilus assembly protein/mating pair stabilization protein (<i>E. coli</i> plasmid F)	0.0; 4.0E-54; 2.0E-08	99/1217; 28/496; 16/595	ZP_00821912.1; ZP_01301957.1; NP_061478.1
144	59876	58488	462	51.27	5.86	Pilus assembly	Hypothetical protein YberA_01001910 (<i>Y. bercovieri</i> ATCC 43970); putative pilus assembly protein (<i>Salmonella enterica</i> sp. enterica serovar Typhi strain CT18); conjugative transfer protein TraH precursor (<i>Shigella flexneri</i> plasmid R100)	0.0; 5.0E-14; 5.0E-11	99/412; 33/164; 26/218	ZP_00821911.1; NP_569448.1; NP_052975.1
145	60749	59886	287	32.57	9.05	Pilus assembly	Thiol-disulfide isomerase and thioredoxins (<i>Y. bercovieri</i> ATCC 43970); conjugative transfer protein TraF precursor (<i>Shigella flexneri</i> plasmid R100)	2.0E-114; 1.0E-20	100/208; 19/248	ZP_00821910.1; NP_052969.1
147	61178	60759	139	14.83	9.28	Pilus assembly	Hypothetical protein YberA_01001908 (<i>Y. bercovieri</i> ATCC 43970); putative lipoprotein (<i>Porphyromonas gingivalis</i> W83)	1.0E-58;	99/132; 32/122	ZP_00821909.1; NP_904997.1
148	61653	61189	154	17.58	7.35	Pilus assembly	Type IV secretory pathway, protease TraF (<i>Y. bercovieri</i> ATCC 43970); TraF protein (plasmid pKJK5)	6.0E-083.0E-73; 3.0E-14	98/137; 20/165	ZP_00821908.1; YP_709182.1
150	62372	61653	239	26.76	8.83	Pilus assembly	Hypothetical protein YberA_01001906 (<i>Y. bercovieri</i> ATCC 43970); TrbC, sex pilus assembly and mating-pair formation protein (<i>Erythrobacter</i> sp. NAP1); conjugative transfer protein TrbC (<i>Shigella flexneri</i> plasmid R100)	4.0E-117; 2.0E-06; 4.0E-10	96/239; 26/205; 19/222	ZP_00821907.1; ZP_01039316.1; NP_052965.1

156	63955	62372	527	58.97	6.03	Conjugative transfer	Hypothetical protein YberA_01001905 (<i>Y. bercovieri</i> ATCC 43970); TraU family protein (<i>Pelobacter propionicus</i> DSM 2379); conjugative transfer protein TraU precursor (<i>Shigella flexneri</i> plasmid R100)	0.0; 2.0E-82; 2e-44 312	99/514; 44/328; 34/312	ZP_00821906.1; YP_899942.1; NP_052963.1
157	64343	64110	77	8.73	4.36	Unknown	Hypothetical protein pYptb0019 (<i>Y. pseudotuberculosis</i> IP 32953)	2.0E-03	32/76	YP_068527.1
162	65969	64359	536	57.69	4.92	Conjugative transfer	Hypothetical protein YberA_01001904 (<i>Y. bercovieri</i> ATCC 43970); essential for conjugative transfer protein (<i>Methylibium petroleiphilum</i> PM1); TraZ (<i>Shigella flexneri</i> plasmid R100)	2.0E-98; 4.0E-24; 1.0E-12	95/184; 38/203; 36/119	ZP_00821905.1; YP_001023391.1; BAA78871.1
164	67995	65944	683	74.84	5.26	Mating-pair stabilization	Hypothetical protein YberA_01001903 (<i>Y. bercovieri</i> ATCC 43970); type IV conjugative transfer system protein TraN (<i>Y. ruckeri</i>)	0.0; 4.0E-25	97/662; 44/149	ZP_00821904.1; YP_001101789.1
167	70570	67997	857	96.95	5.63	Pilus assembly	Type IV secretory pathway, VirB4 components (<i>Y. bercovieri</i> ATCC 43970); sex pilus assembly protein (<i>Syntrophus aciditrophicus</i> SB); conjugative transfer protein TraC (<i>Shigella flexneri</i> plasmid R100)	0.0; 6.0E-142; 4.0E-18	94/849; 34/863; 22/767	ZP_00821903.1; YP_461725.1; NP_052960.1
168	71076	70570	168	18.17	8.17	Unknown	Hypothetical protein YberA_01001901 (<i>Y. bercovieri</i> ATCC 43970)	4.0E-79	98/168	ZP_00821902.1
171	72350	71100	416	44.62	7.10	Pilus assembly/conjugative transfer	Hypothetical protein YberA_01001900 (<i>Y. bercovieri</i> ATCC 43970); TraB pilus assembly family protein (<i>Pelobacter propionicus</i> DSM 2379); conjugative transfer protein TraB (<i>E. coli</i> plasmid F)	3.0E-164; 7.0E-39; 8.0E-10	100/308; 30/413; 23/388	ZP_00821901.1; YP_899948.1; NP_061457.1
173	73135	72347	262	28.68	8.62	Unknown	Hypothetical protein SKA58_02650 (<i>Sphingomonas</i> sp. SKA58)	2.0E-27	33/235	ZP_01301938.1
174	73787	73137	216	24.52	9.12	Pilus assembly	Hypothetical sex pilus assembly and synthesis protein (<i>Sphingomonas</i> sp. SKA58)	1.0E-11	27/176	ZP_01301937.1
175	74069	73797	90	10.76	8.35	Unknown	Hypothetical membrane protein (<i>Sphingomonas</i> sp. SKA58)	2.0E-07	29/89	ZP_01301936.1

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TABLE 5—Continued

ORF	Start position	Stop position	Length (amino acids)	Molmass (kDa)	pI	Predicted function	Related protein(s) and source/description	E value	Extent of best match % identity/length of region (no. of amino acids)	NCBI accession no.
176	74454	74086	122	13.03	8.97	Pilus assembly	Hypothetical protein YberA_01001899 (<i>Y. bercovieri</i> ATCC 43970); conjugative transfer protein TraE (<i>Shigella flexneri</i> plasmid R100)	4.0E-33; 2.0E-04	100/76; 19/181	ZP_00821900.1; NP_052949.1
179	75389	74508	293	32.67	7.36	Isomerase	Protein-disulfide isomerase (<i>Y. bercovieri</i> ATCC 43970)	4.0E-154	96/292	ZP_00821899.1
183	76692	76339	117	13.57	9.39	Terminase	Phage terminase GpA (<i>Shewanella denitrificans</i> OS217)	2.8E-02	32/68	YP_564251.1
185	76792	77067	91	10.52	9.31	Unknown	Hypothetical protein YberA_01001897 (<i>Y. bercovieri</i> ATCC 43970)	1.0E-46	100/91	ZP_00821898.1
187	77277	78677	466	52.42	9.68	Replication	Hypothetical protein pIP404_p03 (plasmid pIP404); replication protein (<i>Clostridium perfringens</i>)	1.0E-06; 5.0E-06	30/153; 31/151	NP_040453.1; YP_209688.1
191	79519	80388	289	31.93	6.33	Partitioning	ATPase involved in chromosome partitioning (<i>Y. bercovieri</i> ATCC 43970); IncC1 partitioning protein (plasmid pB3)	4.0E-160; 9.0E-14	99/274; 26/291	ZP_00821897.1; YP_133955.1
194	80385	81776	463	51.23	5.29	Transcriptional regulator/partitioning	Predicted transcriptional regulator (<i>Y. bercovieri</i> ATCC 43970); plasmid replication/partition protein (<i>Listonella anguillarum</i>); KorbB partitioning and repressor protein (plasmid OKH54)	0.0; 8.0E-10; 2.0E-03	98/463; 30/182; 28/194	ZP_00821896.1; AAO92393.1; YP_619804.1
196	81779	82516	245	28.09	5.39	Unknown	Hypothetical protein LV003 (<i>Klebsiella pneumoniae</i>)	1.0E-05	34/89	NP_943506.1
202	84500	84321	59	7.04	4.63	Unknown	Hypothetical protein RSP_7393 (<i>Rhodobacter sphaeroides</i> 2.4.1)	7.0E-08	57/54	YP_345433.1
205	85265	85888	207	23.36	5.39	Unknown	Hypothetical protein YberA_01003603 (<i>Y. bercovieri</i> ATCC 43970)	2.0E-76	98/142	ZP_00820260.1
207	85889	86638	249	27.85	9.47	Adenylosuccinate synthase	Adenylosuccinate synthase (<i>Y. bercovieri</i> ATCC 43970)	7.0E-111	90/211	ZP_00820259.1
209	87761	86739	340	37.83	9.97	Transposase	Probable transposase (<i>Y. pestis</i> biovar Microtus strain 91001)	0.0	95/340	NP_995565.1
211	88141	89379	412	47.55	9.71	Transposase	Transposase and inactivated derivatives (<i>Y. bercovieri</i> ATCC 43970)	0.0	96/412	ZP_00820226.1
212	88254	87976	92	10.71	9.55	Integrase	Prophage integrase (<i>Erwinia carotovora</i> subsp. <i>atroseptica</i> SCRI1043)	5.0E-06	40/85	YP_050272.1
217	89817	90428	203	23.06	7.48	Unknown	Putative membrane protein (<i>Bordetella avium</i> 197N)	2.0E-23	33/179	CAJ49184.1

219	90628	91551	307	33.54	5.66	Transporter	Cation efflux protein (<i>Dechloromonas aromatica</i> RCB); predicted Co/Zn/Cd cation transporter (<i>Nostoc punctiforme</i> PCC 73102)	1.0E-115; 2.0E-79	70/300; 57/295	YP_284269.1; ZP_00110704.1
223	92449	91808	213	24.83	9.39	Recombinase	Resolvase, N-terminal (<i>Shewanella baltica</i> OS155); site-specific recombinase, DNA invertase Pin homolog (<i>E. coli</i> B7A)	6.0E-98; 3.0E-87	95/210; 94/191	ZP_00580490.1; ZP_00714543.1
224	92617	342	1074	121.49	9.47	Transposase	Transposase (<i>Proteus vulgaris</i>)	0.0	89/1055	NP_639975.1

from all tested pathogenic *Yersinia* strains, from nonpathogenic *Y. enterocolitica* biogroup 1A strain 12, from *E. coli* K12, and from a *Shigella flexneri* strain. Mobilization of p1340 by pYE854-I1/01 occurred with frequencies between 10^{-2} to 10^{-8} per donor cell (Table 4). Southern analysis of plasmids isolated from transconjugants disclosed that, in pathogenic *Y. enterocolitica* strains, the indigenous virulence plasmid was replaced by the newly acquired pYV of *Y. pseudotuberculosis*, discernible by the different restriction patterns of the plasmids (Fig. 1A and C, lanes 5, 6, 8, and 9). DNA hybridization also revealed weak homology between pYE854-I1/01 and p1340 (Fig. 1B, lane 2). It cannot be ruled out that, especially in cases of low transfer frequency, a cointegrate between pYE854 and pYV was formed. However, cointegrate formation has never been observed in the donor strain or any of the analyzed transconjugants. For the mating experiments, we also used as donor an *E. coli* K12 strain in which plasmid p1340 had been introduced by electroporation and pYE854-I1/01 by subsequent conjugation. Even from *E. coli* K12, plasmid p1340 was transmitted to pathogenic *Y. enterocolitica* strains (Fig. 1A and C, lanes 7 and 10), albeit with reduced frequencies (Table 4). Similarly to p1340, pYV of the *Y. enterocolitica* serogroup O:3 strain 13169 (Table 1) was transmitted, a result that documents that the virulence plasmids of both *Y. enterocolitica* and *Y. pseudotuberculosis* can be transferred with the help of pYE854.

Overall sequence analysis of pYE854. The complete nucleotide sequence (95,499 bp) of pYE854 has been determined. The plasmid has an average G+C content of 42.3%, slightly lower than the $48.5\% \pm 1.5\%$ (mean $\pm$ standard deviation) reported for the host *Y. enterocolitica* (5). However, the G+C contents of different ORFs vary in the range from 28% to 60%. The bioinformatic analysis revealed 232 ORFs with potential coding capacity (minimum protein size, 50 amino acids) and an ATG start codon (see Table S2 in the supplemental material for details). Both DNA strands of the plasmid contain an equal number of ORFs. The database search gave matches for 80 deduced gene products with identities to other protein sequences of between 27% and 100% (Table 5). We did not detect genes obviously encoding known virulence factors or genes conferring antibiotic resistance. The strongest overall similarities found were to transfer proteins of *Yersinia bercovieri* and the F plasmid, but significant homologies also exist to proteins belonging to a phosphotransferase system (PTS) of *Vibrio vulnificus*.

Two DNA regions encoding transfer proteins are essential for conjugation. The genetic map of pYE854 shows two regions (Tra1 and Tra2) which probably encode a conjugative transfer system. Similar to IncP plasmids, e.g., RP4 and R751, the Tra regions of pYE854 are separated (Fig. 2). The analysis of the Tra1 region revealed two products (ORF 30 and ORF 36) which are presumably involved in DNA transfer replication and coupling. The putative coupling protein (ORF 30) is related to the TraD of the F factor, which belongs to the TraG family of coupling proteins. It contains motifs that are important for DNA transfer (46). The ORF 36 product might code for a major component of the pYE854 relaxosome. This protein contains sequence motifs that are characteristic for known relaxases and TraI helicases, showing that it belongs to superfamily 1 of the helicases (Fig. 3) (25). Relaxases initiate and terminate conjugation by catalyzing site- and strand-specific

FIG. 2. Genetic map of pYE854. Black bars indicate DNA regions involved in plasmid transmission, replication, and maintenance, as well as genes that might belong to a PTS. The position of the *oriT* is marked by an arrowhead. DNA segments that have been identified and those that are missing in pYE966 are indicated by dashed lines and dotted lines, respectively.

cleavage and rejoining reactions at the plasmid-encoded *nic* site (*oriT*). The origin of transfer is typically located next to the DNA processing genes. Approximately 500 bp upstream of ORF 30, we identified a sequence motif similar to the *oriT* *nic*

region of the F factor (Fig. 4). For functional analyses, a 4.2-kb *Hind*III restriction fragment containing this motif was inserted into the nonmobilizable vector pIV2 (see Materials and Methods). The recombinant plasmid pJH907 was mobilized by

**Helicase Superfamily I
Consensus Sequences**

FIG. 3. Helicase motifs identified within TraI of pYE854 (according to Hall and Matson [25]). *, for the consensus sequence Ia, several possible motifs have been identified (data not shown). The position of the first amino acid residue of each motif of pYE854 is given. aa, amino acids. Amino acid residues identical in motifs of TraI of pYE854 and other helicases are indicated by bold letters. + and O represent a hydrophobic and a hydrophilic residue, respectively. Residues not restricted to being hydrophobic and hydrophilic are represented by an X.

FIG. 4. Isolation of the pYE854 *oriT* region. The upper panel shows the linear gene map of pYE854 and a restriction fragment library (pJH800 series *Cla*I fragments and pJH900 series *Hind*III fragments) used to identify the *oriT* sequences present on pJH808 and pJH907. In the middle, the 4.2-kb *Hind*III restriction fragment of pJH907, which was further reduced in size, is depicted. The smallest DNA fragment conferring mobilization has a size of 309 bp (pJH907-F). +, transfer occurred; –, transfer did not occur. The lower panel illustrates an alignment of the *oriT* sequences of pYE854 and some other, related plasmids. A shaded background marks positions where two different types of nucleotides may occur. Nucleotides conserved within the *oriT* *nic* regions are depicted in bold letters. In cases where the cleavage site has been determined, it is indicated by an arrowhead. On the right, the sequence of the flanking palindrome, which is also essential for conjugation, is shown.

pYE854 with frequencies of up to 10^{-3} per donor, whereas other *Hind*III restriction fragments of a pYE854 library did not mediate mobilization. By stepwise reduction of the 4.2-kb fragment, the functional *oriT* core region was narrowed down to 309 bp (Fig. 4). Adjacent to the *oriT* sequence lies a 30-bp palindrome. Partial removal of the palindrome resulted in a loss of mobilization, demonstrating that the repeat is also essential for plasmid transmission. To exclude the presence of other *oriT* sequences on pYE854 that might have been inactivated by *Hind*III cleavage, a *Cla*I restriction fragment library was examined in terms of mobilization. Only the 3.9-kb restriction fragment of plasmid pJH808 overlapping with the 4.2-kb *Hind*III fragment of pJH907 conferred mobilization of the vector pIV2 (Fig. 4). Hence, pYE854 harbors a single origin of transfer located within the Tra1 region. Since the *oriT* sequence is located on the minus strand of pYE854, DNA transfer is initiated at the left end of the Tra1 region and ends with the transmission of the Tra1 genes.

Besides the aforementioned products, the Tra1 region might code for additional proteins crucial for conjugation. Possible candidates are the products of ORFs 40, 43, and 45, which are

similar to a restriction endonuclease, a lytic transglycosylase, and a DNA-binding protein, respectively, though it remains open which role these proteins might play in DNA transfer.

The Tra2 region is approximately 20 kb in length and contains 17 ORFs whose products show homologies to known proteins (Fig. 2). Eleven of the deduced amino acid sequences are similar to transfer proteins. The majority of the transfer proteins are highly homologous to proteins of *Y. bercovieri* strain ATCC 43970, with identities between 94% and 100% (Table 5). Unfortunately, there is yet no information available about plasmids of this species. Nevertheless, a stretch of 33 kb (pYE854 positions 51500 to 84500) is nearly identical to a published whole-genome shotgun sequence (Y berA_01_29, NCBI accession no. NZ_AALC01000029) of the *Y. bercovieri* strain. This stretch comprises the whole Tra2 region and adjacent DNA sequences of pYE854 (see below). The ORF analysis suggests that, in the Tra2 region, mating-pair formation (Mpf) proteins are encoded. Mpf proteins form a membrane-associated apparatus that synthesizes and assembles mature conjugative pili on the cell surface. The conjugative pili facilitate the initial contact with the recipient cell, resulting in

FIG. 5. Analysis of the pYE854 replication and partitioning (par) region. The upper panel shows miniplasmid derivatives of pYE854 retaining replicative competence. In the middle, the linear gene map of pYE854 is given. The locations of putative centromere sites are indicated. The lower panel shows the sequences of the predicted centromere sites and their positions on the plasmid. Lowercase letters show nucleotides diverging from the dyad symmetry of the palindromes. The vertical arrow and dotted line represent the center of dyad symmetry. Half sides of the palindrome are indicated by horizontal arrows.

the formation of a stable mating pair. The pYE854 products are related to Mpf proteins of several transfer systems, e.g., F and R100 (IncF), R27 (IncH), RP4 (IncP), and R388 (IncW), and of the type IV secretion system of the Ti plasmid of *Agrobacterium tumefaciens*. To determine which of the pYE854 *tra* genes are essential for conjugation, the plasmid was mutagenized by using an in vivo transposon mutagenesis system (see Materials and Methods). The analysis of the insertion sites of the marker gene disclosed that many insertions occurred hot spot-like within transposase genes (data not shown). However, insertions were also found in other ORFs (Table 2). All mutants were investigated in terms of their capability to conjugate and to mobilize pYV. Not surprisingly, insertions leading to a loss of conjugation and mobilization were mainly found in ORFs located in the Tra regions. All seven insertions within the putative *traI* gene (ORF 36) resulted in a deficit of transfer. Within the Tra2 region, several genes supposed to be important for conjugation were mutagenized (Table 2). Insertions in ORF 164 (TraN) only decreased mating ability slightly, consistent with the results of observations of plasmids F and R100-1 (37). All other insertions yielded defective mutants unable to conjugate and to mobilize the *Yersinia* virulence plasmid. In addition to *tra*, insertions resulting

in a negative Tra phenotype were also observed in ORF 122. The function of this gene is not known. Its predicted product shows some similarity to a permease of *Yersinia* belonging to the major facilitator superfamily which comprises translocators exporting antibiotics and other small molecules. The pYE854 replicon belongs to a new incompatibility group. A search for ORFs encoding probable Rep proteins was undertaken to identify DNA regions on pYE854 which might be important for plasmid replication. Two genes (ORF 11 and ORF 187) possibly involved in replication have been detected. While the deduced ORF 11 product shows some similarity to a primase of *Clostridium difficile*, ORF 187, located upstream of the Tra2 region, encodes a protein that is similar (30% identity) to the Rep protein of the *Clostridium perfringens* plasmid pIP404 (Table 5). The *rep* gene of pIP404 is sufficient, in conjunction with some repetitive origin-like sequences located downstream of the gene, to drive plasmid replication (21, 22). We constructed a replicative miniplasmid derivative of pYE854 by digesting the plasmid with PstI and ligating the resulting restriction fragments with a tetracycline resistance gene. Upon transformation of *E. coli* DH5 α , plasmid pJH1010, comprising the 50,189-bp PstI restriction fragment and *tet* (Fig. 5), was isolated. By using other restriction endonucleases, the

size of the minimal replicon could be further reduced. The smallest miniplasmid, pJH1015, is composed of the 8,350-bp NdeI restriction fragment of pYE854 ligated to the tetracycline resistance gene. Besides ORF 187, pJH1015 contains the pYE854 ORFs 183, 185, 191, 194, 196, and 202 (Fig. 5). The relationship of the putative ORF 187 product to a replication protein of *Clostridium* prompted us to investigate whether pJH1015 may replicate in gram-positive bacteria. For that reason, several *Bacillus* strains belonging to various species (see Table S1 in the supplemental material) were transformed with the plasmid. We could not isolate any transformant containing pJH1015, while plasmid pUB110 (36) could be readily introduced into the bacteria, indicating that the pYE854 derivative does not replicate in *Bacillus* species.

To determine the incompatibility group of pYE854, the PCR-based replicon typing scheme developed by Carattoli et al. (6), which covers 18 common plasmid incompatibility groups among the *Enterobacteriaceae*, was applied. Since the recommended primers did not match pYE854, the incompatibility determinants of the reference plasmids were amplified by PCR and the products were hybridized with pYE854. Even by Southern hybridization, pYE854 could not be affiliated to one of the 18 incompatibility groups (data not shown). We also studied incompatibility under in vivo conditions by introducing pYE854 into *E. coli* strains containing plasmids belonging to the Inc groups A, B, C, H, I, J, M, N, P, or Q (data not shown). The *Yersinia* plasmid showed compatibility to all of the investigated replicons. Hence, pYE854 belongs to a new incompatibility group.

Plasmid maintenance is accomplished by a partitioning and an addiction system. Next to the putative *rep* gene of pYE854, there are two ORFs (191 and 194) which apparently belong to a plasmid partitioning system (Fig. 2). Their predicted products are nearly identical to proteins of *Y. bercovieri* and also related to partitioning proteins (Par) of IncP plasmids (Table 5). While the ORF 191 product is similar to ParA-like ATPases, the deduced product of ORF 194 may represent the transcriptional repressor ParB. This protein binds to a centromere target site (*parC*) which consists of repetitive sequences often situated close to the *par* operon. A search for possible centromere sites revealed 14 nearly identical copies of a 16-bp palindromic sequence scattered on pYE854 (Fig. 5). Three copies of this sequence are located upstream of the *parA* gene. This region also contains a number of possible promoter sequences, which suggests that the detected repeats are binding sites for the partitioning proteins and probably involved in the autoregulation of *par* expression.

Stable maintenance of pYE854 might also be achieved by an addiction system (toxin-antitoxin system) responsible for post-segregational elimination of plasmidless cells from a bacterial population. ORFs 15 and 16 apparently encode a toxin and an antitoxin, respectively. Both products are similar to proteins of the bacteriophage N15 that were shown to stabilize heterologous replicons (15). Moreover, overexpression of the N15 toxin gene in *E. coli* resulted in a bacteriostatic effect discernible by elongation of bacterial cells and growth cessation. To find out whether the pYE854 ORF 15 also has adverse effects on bacterial growth, the gene was amplified by PCR and inserted into expression vector pMS470Δ8, which contains an inducible *tac* promoter (3). Upon transformation, the isolated *E. coli* clones were analyzed by sequencing. All plasmids revealed mutations

within ORF 15 leading to amino acid exchanges which probably inactivated the toxin. As the cloned DNA fragment is very small (333 bp), it can be assumed that the intact gene has deleterious effects on the bacteria. This was corroborated by the fact that we could clone PCR fragments containing either the antitoxin gene or both the toxin and antitoxin genes.

Plasmid curing was examined by treating *Y. enterocolitica* strain 29854 with acridine orange at a 0.1 mM concentration. Using this concentration, the plasmid could easily be cured from its original host strain (data not shown).

pYE854 is a cryptic plasmid. As mentioned above, we identified a stretch of genes (ORFs 73, 75, 79, 82, 86, and 89) on pYE854 which obviously code for components belonging to a PTS (Fig. 2, Table 5). A similar cluster exists in *V. vulnificus* YJ016 and also in *Psychromonas* and *Y. pseudotuberculosis*. The products of this gene cluster might be important for the uptake of mannose and fructose. By using an API 50 CHB/E system (bioMérieux, Nürtingen, Germany), we tested if a *Yersinia* strain harboring pYE854 is more capable of utilizing sugars and other carbohydrates than the respective strain without the plasmid. We did not detect any differences between the strains. Thus, it is doubtful whether the gene cluster plays a role in bacterial growth. The same holds true for ORFs 97 and 219, whose predicted products show a relationship to proteins involved in heavy metal resistance (Table 5). A test on agar containing arsenic, cadmium, cobalt, or zinc revealed the sensitivity of the *Yersinia* strains to these elements, regardless of the plasmid content. Therefore, at the present level of knowledge, pYE854 has to be considered cryptic.

pYE966, a pYE854-related plasmid of a pathogenic *Y. enterocolitica* strain. Mating studies conducted with pYE854 showed that this plasmid is transmissible with high frequencies. To find out whether similar plasmids are prevalent in *Yersinia*, we used pYE854 as the probe for DNA hybridization experiments with plasmid preparations of approximately 50 strains of the BfR strain collection. One of the plasmids investigated, pYE966, isolated from pathogenic *Y. enterocolitica* serogroup O:5,27 strain 966/89 (Table 1) displayed strong hybridization signals (Fig. 6). According to the restriction patterns obtained, pYE966 has an approximate size of 70 kb. Two DNA regions of pYE854 are obviously missing in the smaller plasmid (Fig. 2). Using primers deduced from pYE854, genes of plasmid pYE966 coding for a number of transfer proteins, the putative replication protein (ORF 187 product), and the partitioning and addiction systems were amplified and sequenced (data not shown). In addition, the *oriT* region of pYE966 was analyzed. We detected only single mismatches to the pYE854 sequences that did not result in amino acid exchanges, confirming that the plasmids are closely related. The conjugative properties of pYE966 have been studied and compared to those of pYE854. Like its larger relative, pYE966 is a self-transmissible plasmid that is able to mobilize pYV, with slightly higher efficiencies even than pYE854 (Table 4). These findings document that the DNA regions of pYE854 which are missing in pYE966 are apparently not important for plasmid transfer.

DISCUSSION

The *Yersinia* virulence plasmid pYV can be mobilized by conjugative helper plasmids. Allen et al. (2) demonstrated that

FIG. 6. Southern hybridization of pYE854 with pYE966. Several restriction digests of pYE854 were separated on an 0.8% agarose gel and hybridized to labeled pYE966 DNA. Lane M, marker (λ Eco130I); lanes 1, 3, and 5, pYE854; lanes 2, 4, and 6, pYE966.

E. coli strains containing the F factor are able to mobilize pYV of *Y. pestis*. Later on, the DNA sequences located within the low-calcium response (*lcr*) region of pYV were determined to promote plasmid transfer (1). Since the *lcr* region of the plasmid is conserved in the three medically important *Yersinia* species, it can be assumed that the virulence plasmids of *Y. enterocolitica* and *Y. pseudotuberculosis* also possess an *oriT*. However, there is no report on the transmission of pYV of these species. Moreover, mobilization of the virulence plasmid by *Yersinia* donor strains harboring an indigenous conjugative plasmid has not been described yet. As there was a gap of knowledge about self-transmissible plasmids in *Yersinia*, we searched for conjugative plasmids and isolated two similar plasmids, one from a pathogenic and the other from a non-pathogenic *Y. enterocolitica* strain. Both plasmids have the potential to mobilize pYV of *Y. pseudotuberculosis* and also the virulence plasmid of *Y. enterocolitica*. A closely related conjugative plasmid might also be present in *Y. bercovieri* strain ATCC 43970 because more than 50% of the pYE854 DNA sequences, including those encoding transfer and replication proteins, were identified in this species. Thus, a group of related, self-transmissible plasmids is possibly widespread in *Yersinia*. This speculation is underpinned by our data on the transfer properties of the conjugative plasmids. We observed conjugation with high frequencies into a broad range of *Yersinia* strains. The mobilization of pYV was less efficient and restricted to pathogenic *Yersinia* strains and one of the bio-group 1A strains investigated. Therefore, dissemination of the virulence plasmid within *Yersinia* populations (e.g., in pigs) which often comprise pathogenic and nonpathogenic strains cannot be ruled out but does not seem to be very common.

The sequence analysis of pYE854 and pYE966 demonstrated that these plasmids are very similar. They show a relationship to the F factor, especially in the transfer regions, which, unlike the *tra* operon of F, are separated on the *Yersinia* plasmids. In F, four proteins (TraD, TraI, TraM, and TraY) are involved in DNA metabolism during transfer (16). The sequence analysis of pYE854 revealed two products (TraD and TraI) that might be part of the relaxosome of this plasmid.

Counterparts to TraM and TraY of the F factor have not been identified. Interestingly, the *oriT* of pYE854, which is also similar to those of the F group, was identified contiguous to the predicted *traD* gene, while the *oriT* of the F factor lies adjacent to the *traM* gene, approximately 23 kb away from *traD* (50). We have investigated whether F is able to mobilize a recombinant plasmid containing the *oriT* of pYE854 but could not detect any transmission of this plasmid (data not shown). Hence, although some proteins of the pYE854 relaxosome and the *oriT* sequence are similar to F, other transfer proteins and/or binding sites (e.g., for IHF, TraM, or TraY) are apparently too divergent to allow mobilization. We also searched for possible *oriT* sequences on the *Yersinia* virulence plasmid, especially within the *lcr* region. Several families of *oriT* *nic* regions and the *oriT* of pYE854 provided the basis for the search (52). Numerous sequences have been detected all over pYV, but none revealed strong similarity to one of the compared sequences. Therefore, the determination of the *oriT* of pYV requires further experiments, as already performed with pYE854.

Among the deduced pYE854 products belonging to the Mpf system, we found seven proteins showing similarity to pilus assembly proteins (TraB, TraC, TraE, TraF, TraH, TraU, and TrbC) of the F factor (19). Their predicted molecular masses are also in good agreement with those of the corresponding F proteins. The same holds true for two pYE854 products that are related to the mating-aggregate stabilization proteins TraG and TraN of F (17, 41). By contrast, a product similar to the pilus subunit pilin of F is not encoded by pYE854 (18). Taking the data on similarities of the transfer proteins and the *oriT* together, it can be reasoned that pYE854 and pYE966 are F-like plasmids, though this classification does not refer to the replicons of the *Yersinia* plasmids.

We could not allocate the plasmids to a known incompatibility group. Their replicons show some relationship to the *Clostridium* plasmid pIP404 but are obviously not functional in gram-positive bacilli. Stable replication in members of the *Enterobacteriaceae* apparently requires the partitioning system because we were unable to isolate a replicative miniplasmid de-

prived of the *par* operon. Since four identical copies of the predicted centromere sites are situated close to or within the *par* operon, it can be assumed that they are also of importance for miniplasmid maintenance. The stabilization of the native plasmids might be accomplished by additional centromere-like sites scattered on the plasmids and by an addiction system. Interestingly, the identified toxin-antitoxin system is related to that of the temperate *E. coli* phage N15, whose prophage is a linear plasmid (43). Besides pYE854, *Y. enterocolitica* strain 29854 harbors the linear plasmid prophage PY54, which is related to N15 but does not possess an addiction system. This observation raises the question of whether the addiction system of pYE854 was once acquired from PY54.

The isolated conjugative plasmids are large in size and contain numerous ORFs, but at present, they have to be regarded as cryptic. This might be caused by the fact that only 25% of the deduced gene products revealed similarities to known proteins. Apart from *tra* genes and those implicated in plasmid replication and maintenance, the functions of the ORFs could not be assigned. Although genes potentially encoding a PTS or conferring heavy metal resistance have been identified on pYE854, we were not able to demonstrate the respective functions. Perhaps additional genes essential for functionality are lacking on the plasmid, or we did not test for the right compounds (e.g., heavy metals). However, it has also been reported that PTSs are sometimes associated with bacterial virulence (53). The question of whether pYE854 plays a role in the virulence of *Yersinia* cannot be answered at the moment. Besides the PTS genes, the plasmid contains two genes (ORF 62 and ORF 73) whose products are similar to a hyaluronate lyase and a glucuronidase, respectively, of *Vibrio*, proteins which are suggested to be virulence factors of some gram-positive pathogens (34, 40). Nevertheless, the finding that *Y. enterocolitica* 29854 can be cured from its conjugative plasmid without any consequences for growth in broth suggests that pYE854 and, presumably, also pYE966 are not pivotal for the bacteria under laboratory conditions.

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