



Sequencing and annotation of two cryptic plasmids from the fish pathogen *Vibrio salmonicida*

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Introduction

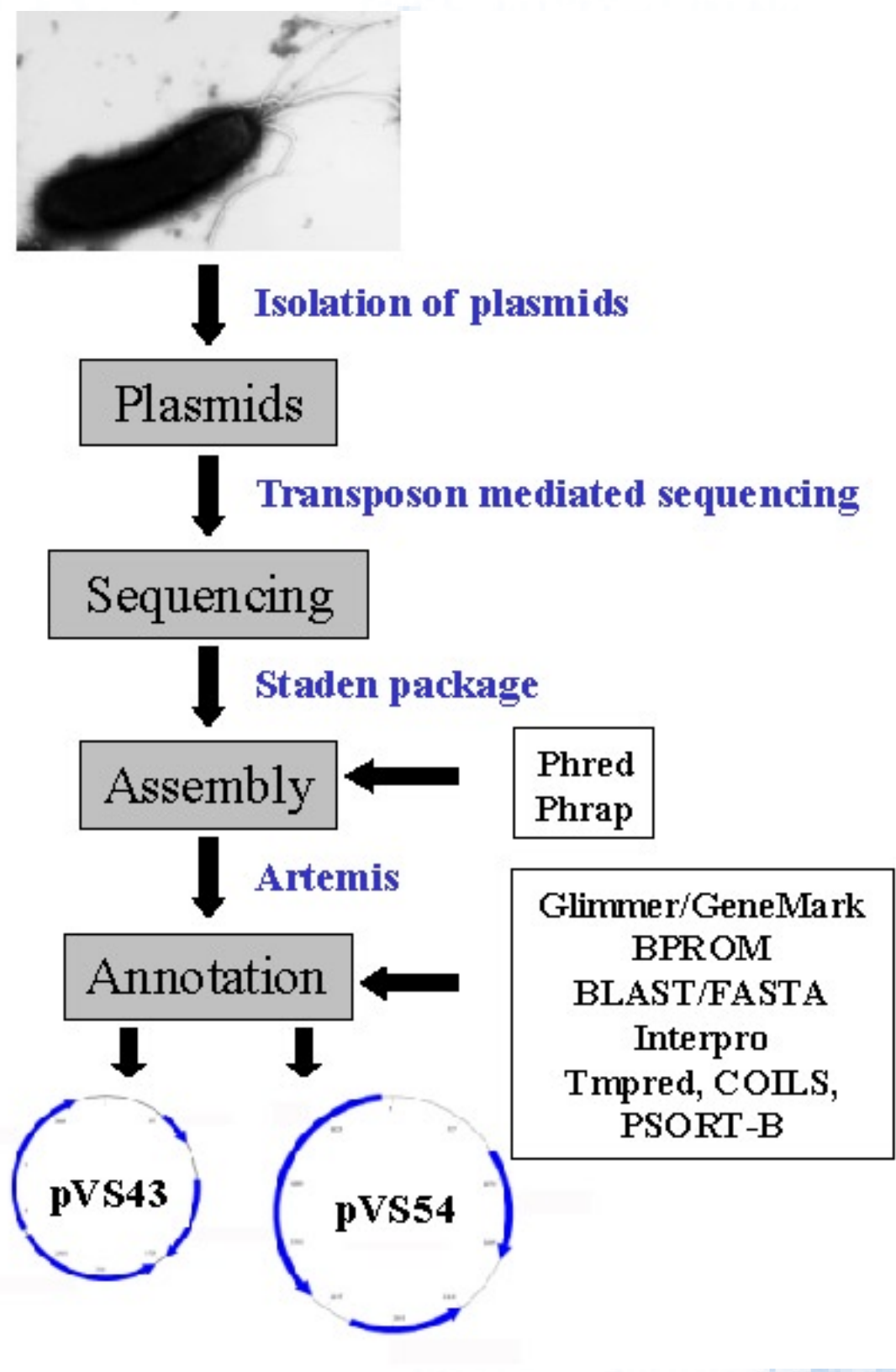
Vibrio salmonicida is responsible for cold-water vibriosis of farmed Atlantic salmon (*Salmo salar*), cod (*Gadus morhua*) and rainbow trout (*Oncorhynchus mykiss*). The bacterium causes tissue degradation, haemolysis and sepsis *in vivo* (Holm *et al*, 1985; Totland *et al.*, 1988). An efficient vaccine controls the disease (e.g. Holm & Jørgensen, 1987; Lillehaug, 1990), but little is known regarding molecular mechanisms of host invasion and specificity, colonisation and growth of the pathogen.

Six plasmids of 170-, 61-, 21-, 5.5/6.72-, 3.4- and 2.8-MDa combined into thirteen different plasmid profiles (Sørum *et al.*, 1988; Wiik *et al.*, 1989; Sørum *et al.*, 1990; Sørum *et al.*, 1993) have previously been identified in several *V. salmonicida* strains.

The intention of this study was to investigate the genetic information encoded by the three cryptic plasmids purified from *V. salmonicida* LFI1238 with respect to possible virulence factors. So far only the two smallest plasmids, pVS43 and pVS54 has been assembled and annotated.

Methods

Figure 1. Schematic presentation of the method part. pVS43 and pVS54 plasmids were isolated and separated. Sequencing was performed on transposon constructs. Assembly was performed using Phred/Phrap in addition to the Staden package. The DNA sequence viewer Artemis was used to annotate the plasmids. The following programs were used to analyse the plasmids: gene prediction: Glimmer2/GeneMark; promoter sites: BPROM; homology searches: BLASTp/FASTA; domain and motifs: Interpro; transmembrane helices: Tmpred; coils: COILS; cellular localization: PSORT-B.



Results & discussion

Isolation of plasmids

The plasmid profile of *V. salmonicida* LFI1238 shows three strong and several weaker bands (Figure 2, B). When the three strongest bands were re-electrophoresed, each gave rise to two other bands on the gel indicating generation of supercoiled and nicked plasmid variants during purification and electrophoresis. The sizes of these cryptic plasmids were estimated to 4.3, 5.4 and 32 kb, respectively (Figure 2).

Plasmids pVS43 and pVS54 (Figure 3) are 4327 and 5360 bp in length, respectively, with G + C contents of 35.6 and 38.1%. The G + C content of the *V. salmonicida* LFI1238 chromosome seems to be 42%. pVS43 and pVS54 both seem to replicate by the theta mode of replication, and pVS43 might belong to the iteron-containing class while pVS54 have origins of replication without iterons (Figure 4).

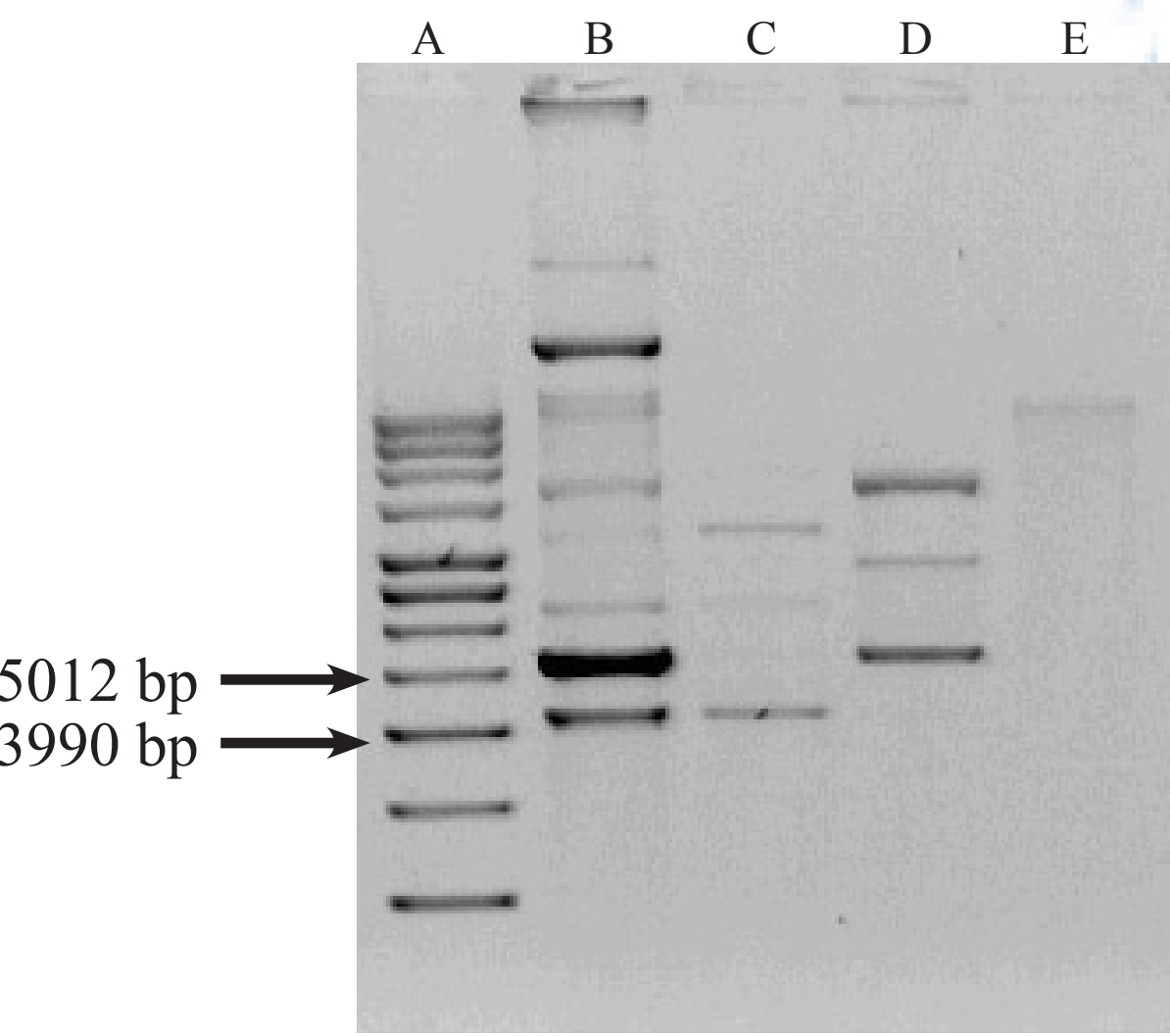


Figure 2. Plasmid profile and separated plasmids of *V. salmonicida* LFI1238. Lanes: A, Supercoiled DNA Ladder (Gibco BRL); B, Plasmid profile; C, pVS43; D, pVS54; E, pVS320 .

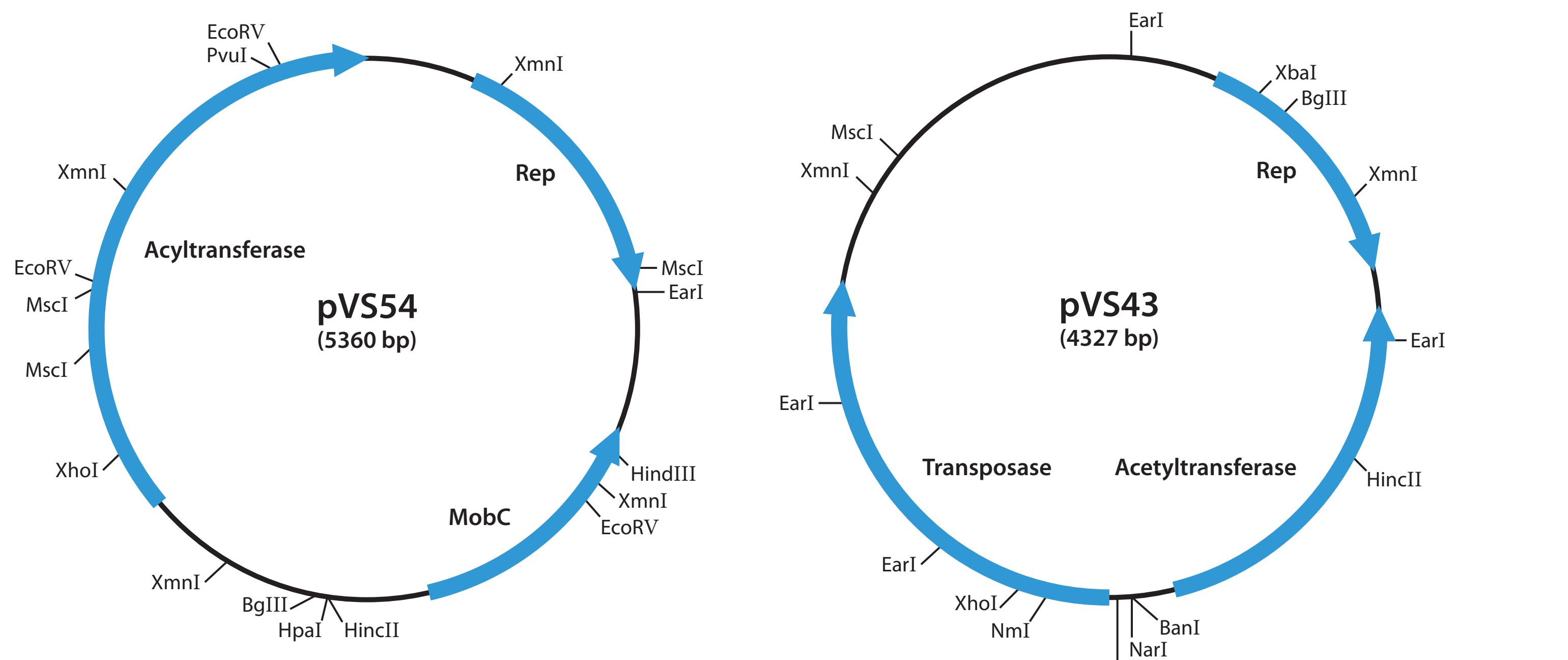
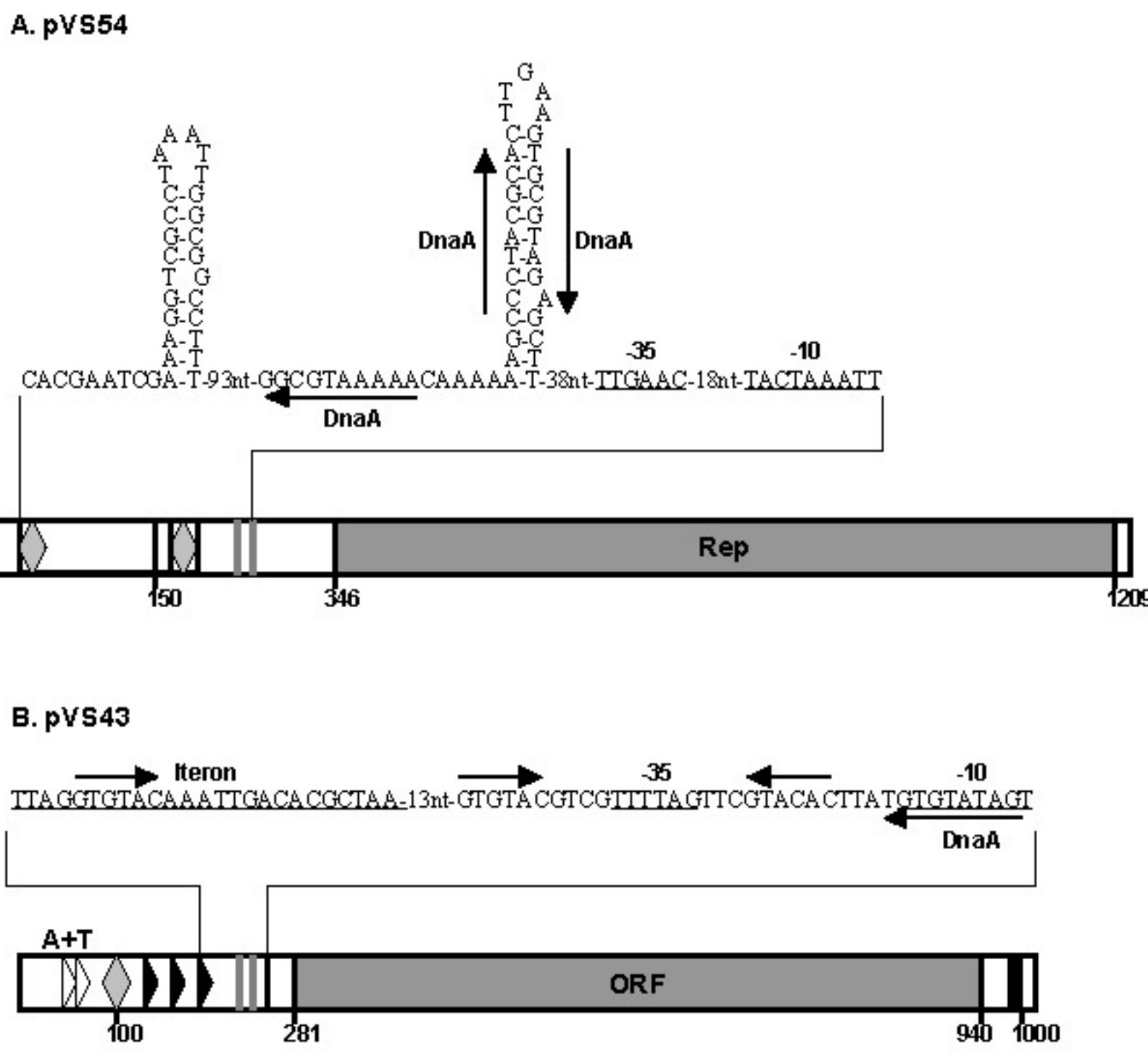


Figure 3. Circular maps of plasmids pVS54 and pVS43. Gene positions with names of putative products and direction of transcription are indicated by arrows. Restriction sites are also marked.

Figure 4. Schematic diagram of the structural organization of the region suggested to be important for replication of plasmid pVS54 (A) and pVS43 (B). The symbols used are as follows: ORF correspond to open reading frame that may encode the Rep protein; closed arrowheads correspond to repeated Rep-binding sequences (iterons); open arrowheads indicate shorter repeated sequences (13-mers); diamond box indicates inverted sequences (in pVS43: palindrome, in pVS54: potential loop forming regions); vertical grey lines correspond to the putative promoters and black box correspond to a putative transcription terminal signal. DnaA boxes are indicated by a vertical black line. The sequence of the cross-hatched region is highlighted where one iteron is represented and underlined; -10 and -35 promoters are indicated and underlined; arrow below the sequence correspond to the DnaA box and arrows above the sequence correspond to inverted repeats that share homology with the iterons.



ORF analysis

The predicted CDS's are presented in Figure 3. Their relationship to homologues in databases and evidences supporting their functions appears in Table 1 and Table 2.

One putative gene in each plasmid might encode for proteins involved in replication. ORF1 in pVS43 shows 45% sequence identity to a replication protein within the iteron-containing class in *Yersinia pestis*, while ORF1 in pVS54 shows weak sequence identity (24%) to *E. coli*.

The gene products of both ORF2 and ORF3 in pVS43 and pVS54, respectively, are thought to be proteins involved in acyltransferase activity. It has been suggested that change in Lipo-polysaccharide (LPS) might lead to an increase in virulence.

ORF3 in pVS43 shows 54 and 46% sequence identity to a putative transposase in *Vibrio anguillarum* and an ISPsy3 transposase in *Pseudomonas syringae*, respectively. Transposases are needed for efficient transposition of the insertion sequence or transposon DNA.

ORF2 in pVS54 shows 25 and 26% sequence identity to a putative mobilisation protein in *Yersinia pestis* and a MobC-like protein in *E. coli*, respectively. MobC in pVS54 might be characterized as a CloDF13 relaxase (Núñez and de la Cruz, 2001).

Table 1. Open reading frames of plasmid pVS54 having significant database matches										
Frame	Codon position (start-stop)	Size aa	G+C content (%)	Molecular mass (kDa)	pl	Putative -10 box	Putative -35 box	Putative protein	Identity to putative protein (%)	Homologous description
ORF1	346-1212 (ATG-TAA)	288	41.7	33.8	9.6	253 TACTAAATT	229 TTGAAC	Replication protein	24	<i>Escherichia coli</i> (UNIPROT Acc.No. Q52221)
								Replication protein	24	Plasmid Collb-P9 (UNIPROT Acc.No. BAA75088)
ORF2	2388-1647 (ATG-TAA)	246	34.8	29.1	10.0	2584 AAAAATAGA	2605 GAAGTT	MobC	25	<i>Yersinia pestis</i> (UNIPROT Acc.No. AA558628)
								MobC	26	<i>Escherichia coli</i> (UNIPROT Acc.No. AAP70929)
ORF3	3432-5360 (ATG-TAA)	642	46.0	72.8	8.2	3393 CTATACAAT	3374 TTGCCT	Acyltransferase family protein	46	<i>Pseudomonas syringae</i> (UNIPROT Acc.No. Q87TX2)
								Acyltransferase family protein	53	<i>Vibrio vulnificus</i> (UNIPROT Acc.No. Q7MPQ5)
								O-antigen	34	<i>Salmonella typhimurium</i> LT2 (UNIPROT Acc.No. Q8ZJN3)
								acetyltransferase		

Table 2. Open reading frames of plasmid pVS43 having significant database matches										
Frame	Codon position (start-stop)	Size aa	G+C content (%)	Molecular mass (kDa)	pl	Putative -10 box	Putative -35 box	Putative protein	Identity to putative protein (%)	Homologous description
ORF1	281-940 (ATG-TAA)	219	31.5	24.8	9.5	253 GTGTATAGT	229 TTTTAG	Replication protein	45	<i>Yersinia pestis</i> (UNIPROT Acc.No. Q9RPN0)
								Replication protein	39	<i>Pseudomonas fluorescens</i> (UNIPROT Acc.No. Q9RLI4)
ORF2	1993-1028 (ATG-TGA)	321	31.3	36.6	9.4	2049 ATCTATAAT	2069 ATGTAT	Acetyltransferase	30	<i>Mesorhizobium loti</i> MAFF303099 (UNIPROT Acc.No. Q986I9)
								O-Acetyl transferase	27	<i>Bacteriophage SF6</i> (UNIPROT Acc.No. P23214)
ORF3	2287-3366 (ATG-TAA)	359	41.5	41.7	9.5	2203 GCTTAAATT	2223 TTTAAT	Putative transposase	54	<i>Vibrio anguillarum</i> (UNIPROT Acc.No. AAR12541)
								ISPsy3 transposase	46	<i>Pseudomonas syringae</i> (UNIPROT Acc.No.Q877Q5)

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