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Nucleotide Sequence of *yst*, the *Yersinia enterocolitica* Gene Encoding the Heat-Stable Enterotoxin, and Prevalence of the Gene among Pathogenic and Nonpathogenic Yersiniae

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The gene encoding the heat-stable enterotoxin (*yst*) was cloned from the chromosome of *Yersinia enterocolitica* W1024 (serotype O:9), and the nucleotide sequence was determined. The *yst* gene encodes a 71-amino-acid polypeptide. The C-terminal 30 amino acids of the predicted protein exactly correspond to the amino acid sequence of the toxin extracted from culture supernatants (T. Takao, N. Tominaga, and Y. Shimonishi, Biochem. Biophys. Res. Commun. 125:845–851, 1984). The N-terminal 18 amino acids have the properties of a signal sequence. The central 22 residues are removed during or after the secretion process. This organization in three domains (Pre, Pro, and mature Yst) resembles that of the enterotoxin ST_a of *Escherichia coli*. The degree of conservation between the *E. coli* and *Y. enterocolitica* toxins is much lower in the Pre and the Pro domains than in the mature proteins. The mature toxin of *Y. enterocolitica* is much larger than that of *E. coli*, but the active domain appears to be highly conserved. The *yst* gene of *Y. enterocolitica* introduced in *E. coli* K-12 directed the secretion of an active toxin. The cloned *yst* gene was used as an epidemiological probe among a collection of 174 strains representative of all *Yersinia* species except *Yersinia pestis* and numerous *Y. enterocolitica* subgroups. In *Y. enterocolitica*, there was a clear-cut difference between pathogenic and nonpathogenic strains: 89 of 89 pathogenic and none of 51 nonpathogenic strains contained *yst*-homologous DNA, suggesting that Yst is involved in pathogenesis. Among the other *Yersinia* species, only four strains of *Yersinia kristensenii* had DNA homologous to *yst*.

Yersinia enterocolitica is an enterobacterium frequently involved in human gastroenteritis, particularly in children (15, 18). The predominant clinical features are abdominal pain, sometimes resembling the pain of appendicitis, and a gastrointestinal disturbance that can vary in its severity from a few loose stools per day to a fulminant enterocolitis with ulcerative lesions involving the entire gastrointestinal tract (37). Bacterial resistance to the primary immune response, leading to mesenteric lymphadenitis, is conditioned by the presence of a 70-kilobase (kb) plasmid called pYV (for reviews, see references 4 and 5).

Y. enterocolitica produces a heat-stable enterotoxin detectable in broth culture supernatant by the infant mouse test (24). This enterotoxin consists of a 30-amino-acid peptide (31). Its physicochemical and antigenic properties as well as its mode of action are similar to those of the heat-stable toxin ST_a (also called STI) of *Escherichia coli* (3, 23, 26). The active site of *E. coli* ST_a appears to be highly conserved in *Y. enterocolitica* enterotoxin (31, 32). We shall refer to the latter as to the Yst enterotoxin.

In view of the close resemblance between Yst and ST_a, it is tempting to speculate that the production of Yst is responsible for the diarrhea associated with yersiniosis. However, two factors argue against this hypothesis: (i) this toxin is detected only in supernatants of cultures incubated at temperatures less than 30°C (8); and (ii) enterotoxin production is common among the *Y. enterocolitica* strains associated with human infections, but it is also encountered in nonpathogenic environmental strains (25).

Hence, at present, the contribution of enterotoxin Yst to the pathogenicity of *Y. enterocolitica* cannot be assessed. The determination of this role awaits experiments conducted with enterotoxin-negative mutants. As a preliminary step to this work, we report here the cloning and the nucleotide sequence of *yst*, the chromosomal gene encoding Yst. We also present data of an epidemiological survey based on DNA-DNA hybridization with the cloned *yst* gene.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* VCS257 is a derivative of strain DP50 (*supF*) used as a host bacterium for in vitro packaging (Gigapack Plus; Stratagene Inc., La Jolla, Calif.). *E. coli* LK111 (C600 Res^- Mod^+ $lacY^+$ $lac\Delta M15$ $lacI^-$) was received from M. Zabeau (Ghent, Belgium). Apart from strain W22703 (serotype O:9), which is the Res^- Mod^+ reference strain that was used to construct the genetic map of pYVe O:9 (6, 22), all the *Yersinia* strains were from the collection of G. Wauters. A derivative of strain W1024 (serotype O:9) cured of its plasmid was obtained by culture at 37°C on MOX agar. MOX agar consists of tryptic soy agar supplemented with 20 mM $MgCl_2$ and 20 mM sodium oxalate.

pLAFR3 is a mobilizable cosmid derived from pLAFR1 (9). The phasmids pBC18R and pBC19R were constructed in our laboratory by cloning the origin of transfer of RK2 (28) into a *Dra*I site of vectors pTZ18R and pTZ19R (Pharmacia, Uppsala, Sweden) (B. China, T. Michiels, and G. Cornelis, Mol. Microbiol., in press).

Growth conditions. For enterotoxin production, bacteria were grown for 48 h at 29°C with shaking in 5 ml of tryptic soy broth (GIBCO Diagnostics, Madison, Wis.) contained in

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a 100-ml flask. The culture was centrifuged for 10 min at 12,000 × *g*, and the supernatant was inoculated into mice.

Biological assay for Yst. Enterotoxic activity in suckling mice was assayed as described by Dean et al. (7). Groups of three 2- to 5-day-old suckling mice were inoculated intragastrically with 0.1 ml of test substance supplemented with 0.1% Evans blue dye as a marker. Mice were kept at 25°C for 3 h and then sacrificed by inhalation of chloroform. The ratio of the intestine weight to the remaining body weight was determined. Values greater than 0.090 were considered positive.

Nucleic acid purification. Plasmid DNA was prepared as described by Balligand et al. (2); chromosomal DNA was prepared essentially as described by Marmur (19).

Construction of a *Y. enterocolitica* chromosomal gene library. Chromosomal DNA from *Y. enterocolitica* W1024, partially digested with *Sau*3A, was fractionated by electrophoresis in a 0.4% agarose gel. Fragments in the range of 18 to 35 kb were electroeluted and used for cloning. Cosmid pLAFR3 DNA was digested to completion with *Bam*HI. The vector and the insert were mixed in a ratio of 10:1 and treated overnight at 7°C with T4 DNA ligase. The total DNA concentration in the ligation reaction was about 250 μg ml^{-1}. The ligation mixture was then packaged into bacteriophage particles with Gigapack Plus (Stratagene) and used to transfect *E. coli* VCS257. Recombinant clones were selected on plates containing tetracycline (10 μg ml^{-1}).

Southern hybridization analysis. Chromosomal or plasmid DNA was digested, electrophoresed, and blotted by standard methods (17, 30). The membrane (Hybond-N; Amersham International plc, Little Chalfont, United Kingdom) was hybridized overnight with 10^6 dpm of the ^{32}P-labeled probe per ml. For the detection of *yst*$^+$ clones in the library, the probe consisted of a mix of eight synthetic 14-mer oligonucleotides (5′-TGGGAA^T_CTG^T_CTG^T_CGA-3′) derived from the protein sequence of Yst. This mix was purchased from Eurogentec S.A. (Liège, Belgium). The probe was radiolabeled with [^{32}P]ATP by using T4 polynucleotide kinase and purified by chromatography through a Sephadex G-50 column (17). Hybridization occurred at 35°C in 5×SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The hybridization temperature (T_{hyb}) was calculated from the following relation (20): $T_{hyb} = T_m\text{min} - 5°C = 4°C \times (G+C) + 2°C \times (A+T) - 5°C$, where $T_m$min is the lowest T_m (melting temperature). Filters were washed twice with constant shaking at the hybridization temperature for 15 min each time in 5× SSC containing 0.1% sodium dodecyl sulfate and once for 10 min in 5× SSC. For the epidemiological survey, the probe consisted of a 212-nucleotide-long *Dra*I-*Hin*dIII fragment (see Fig. 1) purified by electroelution and labeled by nick translation as described previously (21). Hybridization occurred at 65°C in 2× SSC. The membranes were washed at 65°C twice for 15 min in 2× SSC–0.1% sodium dodecyl sulfate and once for 10 min in 2× SSC.

DNA sequencing. The DNA sequence was determined by the dideoxy-chain termination method (27) with a Sequenase kit (United States Biochemical Corp., Cleveland, Ohio).

RESULTS

Cloning of the *Y. enterocolitica* enterotoxin chromosomal gene (*yst*). Several *Y. enterocolitica* strains were tested by the infant mouse assay for their ability to produce the enterotoxin. This test measures the fluid accumulation in the intestine of suckling mice after intragastric injection of enterotoxic material. The results are given in Table 1. Strain W1024 from serotype O:9 was selected to construct a chromosomal library in cosmid pLAFR3. Nine hundred sixty independent *E. coli* VCS257 recombinant clones were obtained. The first screening of the library by colony hybridization using a mix of eight synthetic 14-mer oligonucleotides potentially complementary to the region of the lowest degeneracy (see Materials and Methods) was unsuccessful. Three hundred eighty clones were then screened by the infant mouse test. In a first step, supernatants of cultures grown at 29°C were tested in batches of two (i.e., 0.05 ml of each supernatant per mouse). The vast majority of the recombinant clones yielded a ratio of intestine weight to remaining body weight of less than 0.080. Two batches yielded ratios between 0.080 and 0.090 and were thus suspected to contain a clone producing the enterotoxin. A third batch yielded a ratio greater than 0.090. The six strains of these three batches were tested separately. Three clones that yielded a positive test were tested three times. As shown in Table 1, recombinant clone Nr237 always gave a positive response for Yst activity, while clones NrC62 and Nr202 gave an uncertain response. The test was repeated after culture of these clones at 37°C instead of 29°C, and similar responses were obtained (Table 1).

TABLE 1. Enterotoxin assay of culture supernatants by the infant mouse test

Strain (serotype) or clone	Intestine weight/remaining body weight when cultures grown at[a]:	
	29°C	37°C
Y. enterocolitica		
W1022 (O:3)	0.124 ± 0.010	NT[b]
W1023 (O:3)	0.110 ± 0.006	NT
W1024 (O:9)	0.128 ± 0.015	0.066 ± 0.002
W804 (O:3)	0.099 ± 0.006	NT
W22703 (O:9)	0.073 ± 0.009	NT
E. coli		
Nr237	0.094 ± 0.003	0.104
NrC62	0.084 ± 0.004	0.088
Nr202	0.082 ± 0.006	0.089
VCS257	0.067 ± 0.005	NT

[a] Test considered positive when ratio is greater than 0.090 and negative when ratio is less than 0.080.
[b] NT, Not tested.

The presence of the *yst* gene in clones Nr237 and NrC62 was confirmed by Southern hybridization using the mix of oligonucleotides as a probe. Plasmid pIDC62 from clone NrC62 contained an insert of 23 kb. A Southern blot of an *Eco*RI-*Hin*dIII digest of this plasmid hybridized with the probe at the level of a 3-kb DNA fragment. Plasmid pID237 from clone Nr237 contained an insert of 5 kb and hybridized at the level of a 1.8-kb *Eco*RI-*Hin*dIII fragment. This 1.8-kb fragment is limited by a *Hin*dIII site located in the insert and an *Eco*RI site located within the vector. The restriction map of the chromosomal insert of pID237 is presented in Fig. 1. This latter clone was used for sequencing because of its small size.

Nucleotide sequence of *yst*. The 1.8-kb *Eco*RI-*Hin*dIII fragment of pID237 was subcloned in phasmid pBC19R, yielding pID4. *E. coli* LK111(pID4) gave a negative response in the infant mouse assay, suggesting that *yst* was interrupted at the *Hin*dIII site. Hence, the total 5-kb *Eco*RI-*Pst*I fragment of pID237 was cloned into pBC18R and pBC19R, yielding pID5 and pID6, respectively. *E. coli* LK111(pID6) produced a toxin active in the infant mice. One hundred

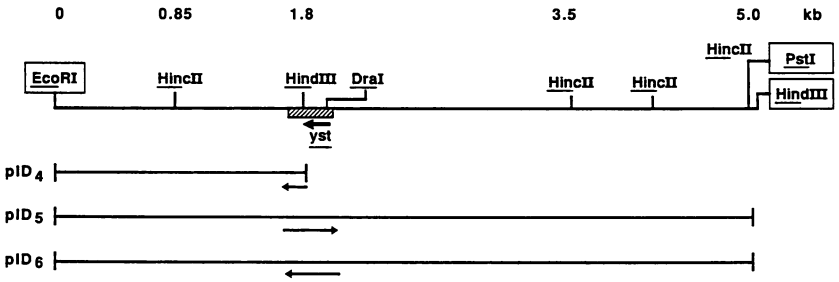

FIG. 1. Restriction map of the chromosomal insert of pID237 and sequencing strategy. The boxed sites are in the vector pLAFR3. The thin arrows give the extent of the sequenced strands, while the thick arrow indicates the direction of transcription of the *yst* gene. The hatched region corresponds to the *yst* gene.

sixty nucleotides encoding the C-terminal amino acid sequence of the enterotoxin (31) were sequenced from the *Hin*dIII site of pID4. The sequence analysis was completed with pID5 and pID6. The strategy of sequencing and the sequence are shown in Fig. 1 and 2, respectively.

The structural gene *yst* consists of an open reading frame of 213 nucleotides starting with an ATG codon at position 117 and ending at position 330 with a TAG stop codon. Three in-phase translational start codons (ATG) occur, at positions 99, 117, and 147. The ATG at position 117 most likely represents the actual start codon for two reasons. First, it is the only one to be preceded by a potential rRNA-binding site (GGAGG) (10). Second, this start codon is the only one to be immediately followed by a potential signal peptide. This peptide is made of the n, h, and c regions (35). The n region, which consists of two lysine residues, is followed by a stretch of nine hydrophobic residues making up a short but nevertheless canonical h region (35). This h region is followed by seven residues ending in Ala-Phe-Gly that constitute a perfect cleavage site according to the (−3,−1) rule (33, 34). These seven residues fulfill the requirements for a c region (35). Possible −35 and −10 RNA polymerase-binding sites occur at positions 59 and 77. The −35 sequence (TTGACA) perfectly fits the −35 consensus sequence of *E. coli* (13). The hypothetical −10 sequence is separated by only 12 nucleotides from the hypothetical −35 box. The *Hin*dIII site known to interrupt *yst* is in position 241.

A comparison of the sequences of *yst* and the *estA1* allele (encoding ST_a) (29), as well as a comparison of their deduced amino acid sequences, is given in Fig. 3. Among 213 nucleotides encompassing codons 1 to 71, there is a sequence divergence of 58%, and only 23 amino acids are conserved. However, despite this high divergence, some local homology occurs between the nucleotide sequences, and their products show a structural similarity. (i) A great degree of homology between *estA1* and *yst* occurs at the 3′ end of the gene; 31 nucleotides out of 39 (80%) are conserved, encoding 11 identical amino acids out of 13 (85%). These 13 C-terminal amino acids constitute the active domain of ST_a (38). An additional amino acid (Tyr) occurs at the COOH terminus of *E. coli* ST_a, but its removal or replacement does not alter the toxicity of ST_a (11). The length of the active extracellular polypeptides differs, however, between Yst and ST_a; for Yst, the secreted peptide is composed of 30 residues, while it only consists of 18 residues for ST_a isolated from pigs (ST_ap) (16) or 19 residues for ST_a from human origin (ST_ah) (1). (ii) Like ST_a (11), Yst possesses a central domain that does not appear in the mature protein (Pro domain). That this domain of ST_a has a role in the export of the toxin across the outer membrane of *E. coli* is likely, but its role has not

```
         6          16          26          36          46          56
      AAACAA TTTAACTATT GGATTTTATT TATTTTTAAA CTCAAAAATT CTTTTTCTGT

        66          76          86          96         106         116
  TATTGACACC ACTGCGTCGA TATTTTTGTA CCCATTCTAC AAATGAGTGA TGGAGGATCT
    -35         -10                                      SD

                                                                   167
ATG AAA AAG ATA GTT TTT GTT CTT GTG TTA ATG CTG TCT TCA TTT GGA GCA
Met Lys Lys Ile Val Phe Val Leu Val Leu Met Leu Ser Ser Phe Gly Ala

                                                                   218
TTC GGC CAA GAA ACA GTT TCA GGG CAG TTC AGT GAT GCA TTA TCG ACA CCA
Phe Gly Gln Glu Thr Val Ser Gly Gln Phe Ser Asp Ala Leu Ser Thr Pro

                                                                   269
ATA ACC GCT GAG GTA TAC AAG CAA GCT TGT GAT CCT CCG TCG CCA CCA GCC
Ile Thr Ala Glu Val Tyr Lys Gln Ala Cys Asp Pro Pro Ser Pro Pro Ala

                                                                   320
GAA GTC AGT AGT GAT TGG GAT TGC TGT GAT GTA TGT TGC AAT CCC GCG TGT
Glu Val Ser Ser Asp Trp Asp Cys Cys Asp Val Cys Cys Asn Pro Ala Cys

            332         342         352
GCT GGC TGC TAG GCATAAATAG CATCAATAGT
Ala Gly Cys
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FIG. 2. Nucleotide sequence of the *yst* gene. The hypothetical −35, −10, and Shine-Dalgarno (SD) (10, 13) sequences are underlined. Boxes indicate the three potential in-phase translational start codons.

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                                                                   167
ATG AAA AAG ATA GTT TTT GTT CTT GTG TTA ATG CTG TCT TCA TTT GGA GCA
Met Lys Lys Ile Val Phe Val Leu Val Leu Met Leu Ser Ser Phe Gly Ala
Met Lys Lys Leu Met Leu Ala Ile Phe Ile Ser Val Leu Ser Phe Pro Ser
ATG AAA AAG CTA ATG TTG GCA ATT TTT ATT TCT GTA TTA TCT TTC CCC TCT

                                                                   218
TTC GGC CAA GAA ACA GTT TCA GGG CAG TTC AGT GAT GCA TTA TCG ACA CCA
Phe Gly Gln Glu Thr Val Ser Gly Gln Phe Ser Asp Ala Leu Ser Thr Pro
Phe Ser Gln Ser Thr Glu Ser Leu Asp Ser Ser Lys Glu Lys Ile Thr Leu
TTT AGT CAG TCA ACT GAA TCA CTT GAC TCT TCA AAA GAG AAA ATT ACA TTA

                                                                   269
ATA ACC GCT GAG GTA TAC AAG CAA GCT TGT GAT CCT CCG TCG CCA CCA GCC
Ile Thr Ala Glu Val Tyr Lys Gln Ala Cys Asp Pro Pro Ser Pro Pro Ala
Glu Thr Lys Lys Cys Asp Val Val Lys Asn Asn Ser Glu Lys Lys Ser Glu
GAG ACT AAA AAG TGT GAT GTT GTA AAA AAC AAC AGT GAA AAA AAA TCA GAA

                                                                   320
GAA GTC AGT AGT GAT TGG GAT TGC TGT GAT GTA TGT TGC AAT CCC GCG TGT
Glu Val Ser Ser Asp Trp Asp Cys Cys Asp Val Cys Cys Asn Pro Ala Cys
Asn Met Asn Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys
AAT ATG AAC AAC ACA TTT TAC TGC TGT GAA CTT TGT TGT AAT CCT GCC TGT

                332
GCT GGC TGC TAG          yst gene
Ala Gly Cys              enterotoxin ST of Y. enterocolitica (Yst)
Ala Gly Cys Tyr          enterotoxin STap of E. coli
GCT GGA TGT TAT TAA      estAI gene
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FIG. 3. Comparison between the open reading frames of genes *yst* and *estA1* (29) and their amino acid products. Identical amino acids are indicated by boxed regions. Symbols: ▒, Pre domain; ■, Pro domain; ■, mature toxin.

TABLE 2. Prevalence of *yst* in yersiniae: hybridization with a *yst* probe

Species	O serotype	No. of tested strains	No. of *yst*$^+$ strains
Pathogenic *Y. enterocolitica*	1	1	1
	1,2a,3	1	1
	2a,2b,3	1	1
	3	35	35
	5,27	8	8
	9	28	28
	4	2	2
	8	4	4
	13a,13b	3	3
	18	1	1
	20	2	2
	21	3	3
Nonpathogenic *Y. enterocolitica*	5	14	0
	6,30	5	0
	6,31	3	0
	7,8	10	0
	10	1	0
	10,34	1	0
	13,7	3	0
	14	4	0
	16	1	0
	16a,58	4	0
	22	1	0
	41,43	1	0
	47	3	0
Y. pseudotuberculosis	IB	1	0
Y. frederiksenii	5-16a,58-48	7	0
Y. bercovieri	8-10-16a,58	7	0
Y. kristensenii	11	2	2
	12	2	2
	16	1	0
Y. intermedia	40-48-52,53	5	0
Y. mollaretii	6,30,47-22-59	6	0
Y. aldovae	21-NT-52	3	0

yet been determined. Yst and ST_a are only 27% identical in this Pro domain. (iii) At the NH_2 terminus (Pre domain), the identity between ST_a and Yst is only 6 of 18 amino acids. However, this domain of Yst represents a correct signal peptide. The fact that Yst was secreted by *E. coli* harboring the recombinant clone reinforces the assumption that Yst, like ST_a (12), contains a Pre domain having the properties of a signal peptide.

Hybridization of *yst* to DNA from *Yersinia* strains. A total of 174 *Yersinia* strains (1 *Y. pseudotuberculosis*, 140 *Y. enterocolitica*, 7 *Y. frederiksenii*, 7 *Y. bercovieri*, 5 *Y. kristensenii*, 5 *Y. intermedia*, 6 *Y. mollaretii*, and 3 *Y. aldovae*) were examined for the presence of *yst*-homologous DNA. These strains originated from various parts of the world and have been collected over a period of more than 10 years (a complete listing of the strains used can be obtained by request from G. Cornelis). In the context of this study, *Y. enterocolitica* serotypes O:1, O:1,2,3, O:2,3, O:3, O:5,27, and O:9 (generally isolated outside the United States) and serotypes O:4, O:8, O:13a,13b, O:18, O:20, and O:21 ("American" strains) are designated pathogenic. Serotypes which are not ordinarily associated with clinical disease (see reference 4 for a review) are designated nonpathogenic. Chromosomal DNA was extracted from all the strains and digested with *Hin*cII, and Southern blot analysis was performed with a DNA probe encompassing part of the *yst* gene

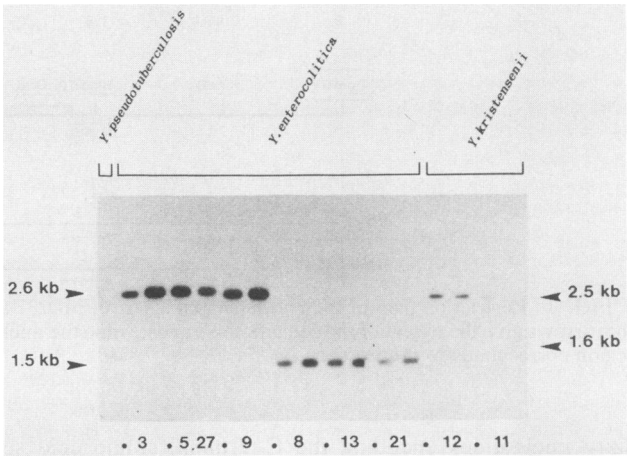

FIG. 4. Different patterns observed in Southern analysis with a *yst* probe. The numbers at the bottom of the figure indicate the serotypes of the tested strains. For each serotype two strains are presented; the sizes are given in kilobases. For *Y. kristensenii* serotype O:11, the hybridizing fragment occurring at the level of 1.6 kb can barely be seen.

(nucleotides 34 to 246 of the sequence given in Fig. 2). The results are given in Table 2. As can be seen, 89 of 89 pathogenic strains had DNA homologous to *yst*. It is noteworthy that strain W22703 also contains *yst*-homologous DNA in spite of the fact that it is enterotoxin negative according to the suckling mouse test (Table 1). Interestingly, we observed only two different patterns: all the American strains had a 1.5-kb fragment that hybridized with *yst*, while the other strains hybridized at the level of a 2.6-kb fragment (Fig. 4). Of 51 nonpathogenic *Y. enterocolitica* strains, none had DNA homologous to *yst*.

Among 34 *Yersinia* strains from other species, only 4 contained *yst*-homologous DNA. These four strains belong to the species *Y. kristensenii*. Three of them hybridized at the level of a 2.5-kb fragment, and the fourth hybridized weakly at the level of 1.6 kb (Fig. 4).

DISCUSSION

In this study we present the cloning of the chromosomal gene *yst* which encodes the *Y. enterocolitica* heat-stable enterotoxin. The screening was based on the biological activity in suckling mice. We showed that the *yst* gene comprises an open reading frame of 71 codons. The COOH portion of the deduced protein corresponds to the 30-amino-acid enterotoxin described by Takao et al. (31). This portion probably represents the active extracellular enterotoxin of *Y. enterocolitica*.

The fact that *yst* governed the secretion of an active toxin in *E. coli* culture supernatants indicates either that the cloned *yst* gene contains sufficient information for the secretion process or that some export factors similar to those of *Y. enterocolitica* are present in *estA*-negative *E. coli*. The secretion of Yst and ST_a most probably occurs through the same mechanism, and the understanding of this process remains a challenge.

In *Y. enterocolitica*, the product of Yst is dependent on temperature (8); the toxin is not detected in supernatants from cultures grown at temperatures of greater than 30°C. Introduced into *E. coli*, the *yst* gene encoded an active enterotoxin at 37°C as well as at 29°C. This suggests the existence of a *Y. enterocolitica* thermoregulator gene that is

absent in our cloned fragments and in the *E. coli* host. A similar phenomenon occurs for the *inv* gene in *Y. pseudotuberculosis* (14). One can hypothesize that a chromosomal regulator gene common for *yst* and *inv* exists. The construction of hybrid operons between *yst* and a selectable gene devoid of its promoter could allow this hypothesis to be tested.

The role of the enterotoxin Yst in the pathogenicity of *Y. enterocolitica* remains obscure. The knowledge of the nucleotide sequence of *yst* will help clarify this role. The sequence allowed us to construct a probe and to search for *yst* in 174 clinical isolates. Pai et al. (25) had previously tested 414 *Y. enterocolitica* strains for the presence of the heat-stable enterotoxin by the suckling mouse test. According to these authors, the production of an enterotoxin is the rule (99%) among pathogenic *Y. enterocolitica* strains but is also common (39%) among nonpathogenic strains. Our study based on DNA-DNA hybridization confirmed that *yst* is present in 100% of the pathogenic strains but revealed that *yst* is absent in nonpathogenic strains. Two reasons may account for this discrepancy. First, the biological test could reveal the presence of an enterotoxin different from Yst in nonpathogenic strains such as O:5, O:6,30, O:6,31, O:7,8, O:13,7, and O:16. Second, the taxonomy of yersiniae has been refined since 1978, and some serotypes such as O:18 and O:21 are now known to contain pathogenic and nonpathogenic strains. Moreover, the species *Y. mollaretii* and *Y. bercovieri* were described in 1988 (36). Previously, these species were considered to be *Y. enterocolitica*. Hence, we believe that the prevalence of Yst among nonpathogenic strains must be reassessed in view of the hybridization data. The fact that DNA homologous to *yst* is regularly detected among the pathogenic strains and not among the others supports the hypothesis that Yst is a virulence determinant. In this case, the presence of *yst*-homologous DNA in some *Y. kristensenii* strains should incite clinical microbiologists to look for a link between the isolation of these strains and diarrhea. Finally, this sequence will facilitate the construction of *yst*-negative strains by in vitro manipulations and allelic exchange. It will then be possible to compare the pathogenicity of a pair of *yst*-positive and *yst*-negative strains in animal model.

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