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IDENTIFICATION AND MAPPING OF THE *SALMONELLA* ENTEROTOXIN GENE

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Mei-Kwei Yang, Hong-Dar Wang and Yen-Chun Yang (1992) Identification and mapping of the *Salmonella* enterotoxin gene. *Bull. Inst. Zool., Academia Sinica* **31**(3): 169-180. *Salmonella typhimurium* strains were isolated from the feces of patients with diarrhea by screening with a DNA hybridization probe encoding *Escherichia coli* enterotoxin (LT). By using LT-A and LT-B as probes, the *S. typhimurium* enterotoxin gene was located on the 5.2-kilobase (kb) pair *Hind* III region of plasmid DNA from strain 22. Recombinant plasmid pST2252 containing this DNA fragment was constructed. Nine restriction enzymes—*Xba* I, *BamH* I, *Hinc* II, *Pvu* I, *Cla* I, *Ava* II, *Puv* II, *Sac* I, and *Acc* I—were selected, and a detailed physical map of the 5.2 kb *Hind* III fragment was constructed. A Southern blot analysis demonstrated that the LT-like enterotoxin gene of *S. typhimurium* was located between the *Acc* I and *Pvu* I sites.

The 2.5-kilobase (kb) *Acc* I-*Pvu* I pair fragment of pST2252 was cloned onto pBluescript II KS (+) and pBluescript II SK (+) vectors, yielding two recombinant plasmids (pST2501 and pST2502) with different orientations. Two polypeptides with appropriate molecular weights of 26 kD and 15 kD, encoded by the 2.5 kb inserted sequence, were identified in *E. coli* minicells. Crude cell lysates of *E. coli* DH5α (pST2502) containing the cloned *Salmonella* LT-like toxin gene showed apparent fluid secretion in a rabbit ileal loop and increased vascular permeability in a skin test. These results clarified the possible role of *S. typhimurium* enterotoxin in the pathogenesis of diarrhea.

Key words: Restriction map, LT-like toxin, *Salmonella typhimurium.*

Salmonella-mediated gastroenteritis is a common and important human disease. Ingested organisms adhere to, penetrate, and damage the mucosal cells of the intestine, thereby causing inflammatory diarrhea. Abnormal fluid secretion is produced due to the destruction of absorptive cells or alterations in intestinal functions (Peterson *et al.*, 1984). The production of bacterial toxins and the invasion of host tissue are the two most important events involved in the pathogenesis of *Salmonella* gastroenteritis. Increases in intracellular concentrations of cyclic adenosine monophosphate (cAMP) suggest that *Salmonella*-mediated diarrheal disease may be similar to symptoms involved by cholera enterotoxin (CT) of *Vibrio cholerae*, or heat-labile enterotoxins (LTs) of *Escherichia coli* (Giannella *et al.*, 1973, 1975). Both enterotoxins are closely related in structure, mechanism of action, and immunochemistry (Clements and Finkelstein, 1978; Hank and Kaper, 1983; Hewlett *et al.*,

1974; Smith and Sack, 1973; Spicer and Noble, 1982). They are composed of one A subunit and five B subunits, and possess the ADP-ribosylating activity of the Gs subunit of adenylate cyclase. The genes for LT and CT have been cloned and sequenced, and a high degree of homology shows that they are closely related (Yamamoto *et al.*, 1984). A similar adenylate cyclase that stimulates an LT-like enterotoxin has also been isolated and characterized from *Salmonella typhimurium* (Finkelstein *et al.*, 1983; Sandefur and Peterson, 1976; Sedlock and Deibel, 1978). However, the precise pathogenic mechanism of this enterotoxin has not been determined.

In an attempt to understand the molecular nature of *Salmonella* enterotoxin, *S. typhimurium* strains isolated from the fecal specimens of humans with diarrhea were tested for the presence of the enterotoxin. A heat-labile toxin which caused the rounding of cultured mouse Y-1 adrenal cells, an elongation of cultured CHO cells, and an increase in the vascular permeability of rabbit skin suggested that the biological activities of the toxin produced by *S. typhimurium* were similar to those of *E. coli* (LT) (Koupal and Deibel, 1975; Moss and Richardson, 1978; Peterson and Sandefur, 1979). Neutralization tests with rabbit ileal loops demonstrated that toxicity was completely neutralized by anti-LT-I antiserum. Similar *Salmonella* LT (S-LT) was isolated from *S. typhimurium* by Finkelstein and his associates in 1983. They demonstrated that S-LT had subunit structures similar to CT and LT. Based on these observations, we performed colony hybridization using radio-labelled DNA probes on the structural genes of the A and B subunits of LT-I to determine the presence of structure genes for S-LT (Yang and Tan, 1989). We found that the DNA sequences involved in the toxin production are located on a 5.2 kb *Hind* III fragment of a plasmid carried by *S. typhimurium* strain 22. In order to examine the structural organization in detail, we described the subcloning and identification of the DNA sequence which is essential for the production of the LT-like toxin. In addition, polypeptides encoded by this cloned DNA fragment were expressed and analyzed in minicells.

MATERIALS AND METHODS

Bacterial strains and plasmids

E. coli HB 101 (pEWD 299) containing a multicopy recombinant plasmid encoding LT was obtained from Dr. C. H. Lee, Department of Pathology, Indiana University, USA. This minicell-producing *E. coli* strain was described by Smits and his associates in 1978. Plasmid pST 2252 consists of a 5.2-kilobasepair (kb) *Hind* III fragment which carries the enterotoxin gene of *S. typhimurium* strain 22 inserted into the *Hind* III site of pUC 19. The plasmid pEWD 299 was described by Dallas *et al.* in 1979. Two plasmids—pBluescript II KS (+) and SK (+)—were used as vectors for subcloning as described by Yanisch-Perron *et al.* (1985).

Restriction enzyme mapping

The purified plasmid DNA was isolated using procedures described by Birnboim and Doly (1979). Restriction endonuclease digestion was carried out as described by Sambrook *et al.* (1989). Restriction enzymes used were obtained from Bethesda Research Laboratories, Inc., Gaithersburg, MD,, and Promega Corp., Madison, WI., and were used in accordance with supplier recommendation. Restriction fragments were analyzed by electrophoresis through 0.7% agarose gels.

Recombinant DNA methods

The methods used for constructing and manipulating recombinant DNA molecules were described by Sambrook *et al.* (1982). A given plasmid and the pBluescript II KS (+) and SK (+) vectors were digested with an appropriate restriction enzyme; the resulting fragments were separated by agarose gel electrophoresis, then eluted from the gel. After ligation, the mixture was transferred into *E. coli* DH5α. The identity of clones was confirmed by restriction digestion and hybridization.

Southern blot hybridization

The standard procedures for agarose gel electrophoresis and the transfer of DNA fragments to nitrocellulose paper were performed as described by Southern (1975). Two *Xba* I-*EcoR* I fragments with 0.77 kb and 0.65 kb from pEWD 299 (Dallas *et al.*, 1979) containing *E. coli* LT A and B subunit genes were isolated by agarose gel electrophoresis. The probes were labelled with [α-^{32}P] dCTP (Amersham International Plc., England) to a specific activity of $10^8 \sim 10^9$ cpm μg^{-1} using a multiprimer DNA labelling kit (Amersham International Plc., England).

Protein analysis in *E. coli* minicells

Plasmid-encoded proteins were examined in *E. coli* minicells as described by Smits *et al.* (1978). *E. coli* Eco 867 containing the appropriate plasmid was grown with shaking in 500 ml L-broth containing antibiotics at 37°C. Cells were harvested by centrifugation at 15,000 xg for 10 min., then resuspended in 20 ml buffered saline gelatin (BSG) containing 0.85% NaCl, 0.03% KH_2PO_4, 0.06% Na_2HPO_4, and 100 μg/ml gelatin, then cultured again by centrifugation. Pellets were resuspended in 1 ml of BSG, loaded on top of 20–25 ml sucrose gradients in a centrifuge tube, and centrifuged for 15 min at 4,000 xg. The minicell fraction was drawn, washed with BSG by centrifugation, and purified by centrifugation again in a sucrose gradient. Preincubation of the minicells in 1 ml of Methionine Medium containing 0.5 mM $MgCl_2$, 0.6 mM $CaCl_2$, 0.25% glucose, and 1 mg/ml thiamine at 37°C for 45 min. was performed, followed by further incubation with 40 μ Ci of L-[^{35}S] methionine for 90 min. The minicell pellet was collected by centrifugation, suspended in 5 ml L-broth, and incubated for 10 min. at 37°C. The [^{35}S] methionine-incorporated minicells were collected by centrifugation, analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and identified by both Coomasic Blue staining and autoradiography.

Rabbit ileal loop assay

Assays for enterotoxic activity were performed in a rabbit ileal loop as described by Sedlock and Deibel (1978). Ten-to-twelve 5 cm-long intestinal loops were tied in rabbits; 1.0 ml duplicated samples were tested and injected in appropriate positions. Ileal loops were injected with 0.2 ml of PD buffer (NaCl 8 g/l, KCl 0.2 g/l, Na_2HPO_4 1.15 g/l, and KH_2PO_4 0.2g/l) (Peterson *et al.*, 1979), and Tryptic Soy Broth (TSB) medium (Gibco: peptone 140, 14 g/l; peptone 110,3 g/l; Dextrose, 2.5 g/l; NaCl, 5 g/l; K_2HPO_4, 2.5 g/l) as negative controls.

Rabbit skin permeability test

Enterotoxin production was also confirmed by rabbit skin permeability test as described by Sandefur and Peterson (1976). The skin of an adult New Zealand albino rabbit was shaved, 0.1 ml of the test sample was injected intradermally. After 18 to 24 h, zones of firm induration were observed and estimated.

RESULTS

Our preliminary experiments with the LT gene probe (*Xba* I-*EcoR* I fragment of plasmid pEWD 299) demonstrated that the *S. typhimurium* enterotoxin gene locates on plasmid DNA and not on chromosomal DNA (Yang and Tan, 1989). On the basis of Southern blot hybridization, we found that DNA fragments with sizes of 5.2 kb of strain 22, 5.0 kb of strain 52, and 8.6 kb of strain 59 contained the LT-like enterotoxin gene. This observation prompted us to examine how these toxin genes were related at the DNA level. The 5.2 kb *Hind* III fragment of *S. typhimurium* strain 22 was subsequently used in our cloning experiment.

Restriction enzyme analysis

We screened 24 different restriction enzymes to match the number of sites present on the 5.2 kb *Hind* III DNA fragment of plasmid isolated from *S. typhimurium* strain 22; we then selected 9 enzymes (*Xba* I, *BamH* I, *Hinc* II, *Pvu* I, *Cla* I, *Ava* II, *Pvu* II, *Sac I*, and *Acc* I) for detailed mapping studies. There are unique *Xba I*, *BamH* I, *Hinc* II, *Pvu* I, *Cla*

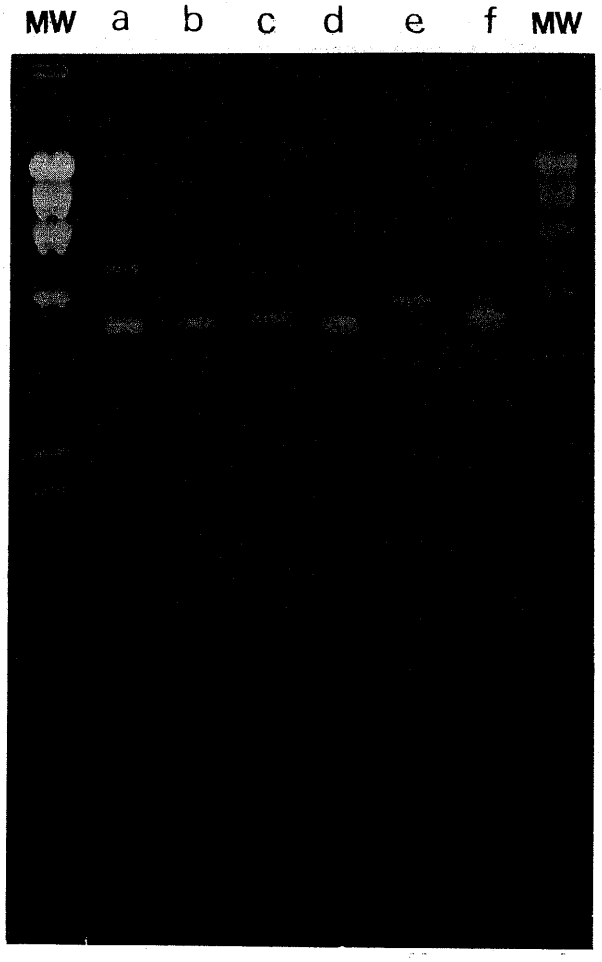

Fig. 1. Agarose gel electrophoresis of plasmid pST2252 digested with restriction endonucleases. Lane a, *Xba* I; Lane b, *Xba* I and *BamH* I; Lane c, *BamH* I; Lane d, *Xba* I and *Hinc* II; Lane e, *Hinc* II; Lane f, *Hinc* II and *BamH* I. Lambda DNA digested with *Hind* III was used as molecular weight marker (MW).

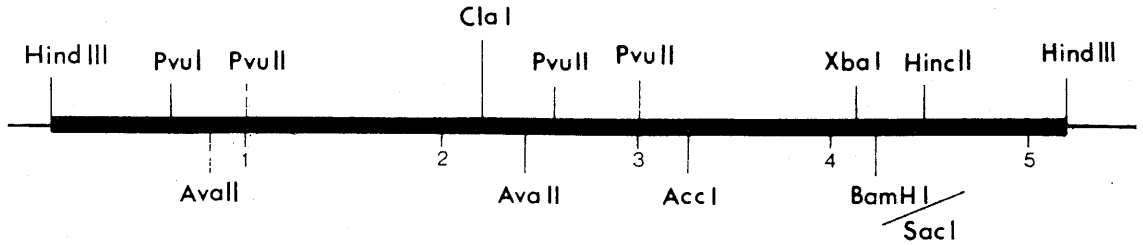

Fig. 2. Restriction endonuclease cleavage map of the 5.2 kb *Hind* III DNA fragmet of hybrid plasmid pST2252. Numbers indicated below the map represent size of region in kilobases.

I, and *Acc I* sites, two *Ava* II sites, and three *Pvu* II sites. Digestion with *Xba* I resulted in two fragments of 4.1 kb and 1.1 kb (Fig. 1a). The *Xba* I site was localized for constructing the restriction maps shown in Fig. 2. A single digest of pST2252 with *BamH* I was run in parallel with a double digest of pST2252 with *Xba* I and *BamH* I (Fig. 1b, c). The 1.1 kb *Xba* I fragment was cleaved by *BamH* I to a 1.0 kb fragment, showing that the *Xba* I and *BamH* I sites are quite close to each other. Similar parallel single and double digests that oriented *Xba* I with *Hinc* II, *Pvu* I, *Cla* I, *Acc I*, *Sac* I, *Ava* II and *Pvu* II have previously been observed. Restriction with *Ava* II resulted in three fragments of 2.7 kb, 1.6 kb, and 0.9 kb. The *Ava* II site was determined by comparison with the digestion of pST2252 with *Ava* II and *Cla* I, as shown in Fig. 2. A similar strategy was employed to localize the *Pvu* II site.

Localization of the LT-like enterotoxin gene

To locate and map the LT-like enterotoxin gene in *S. typhimurium*, we used the *EcoR* I-*Xba* I 0.77 kilobase (kb) and 0.65 kilobase (kb) pair DNA fragments from plasmid pEWD 299 (Dallas *et al.*, 1979), labelled with ^{32}P to a high specific activity, as hybridization probes. The 0.77 kb fragment contains the LT-A subunit gene, and the 0.65 kb fragment contains the LT-B subunit gene. Plasmid DNA from *S. typhimurium* strain 22 was cut with *Hind* III, and a 5.2 kb hybridizing band resulted. After isolating this fragment and cutting it with various types of restriction endonuclease, the resulting fragments were separated by agarose gel electrophoresis and blotted onto nitrocellulose paper. The blots were hybridized and subjected to autoradiography. As shown in Fig. 3, of the two 4.1 kb and 1.1 kb fragments generated by *Xba* I digestion, only the 4.1 kb fragment hybridized with both the LT-A and LT-B probes; it appears that 1.1 kb *Xba* I fragment did not contain the LT-like toxin gene. The 4.1 kb *Xba* I fragment was then subjected to *Acc* I digestion, and two fragments of 3.2 kb and 0.9 kb were produced; we found that only the 3.2 kb fragment hybridized with the probe. This indicates that the sequences on the right side of *Acc* I (Fig. 3) are not related to the LT-like toxin gene. We supposed that the 3.2 kb *Xba* I-*Acc* I DNA fragment contained the LT-like enterotoxin gene.

Further restriction enzyme digestion was performed to find the actual location of the LT-like gene. After digesting the 3.2 kb *Xba* I-*Acc* I fragment with *Pvu I* and *Cla* I, 1.7 kb, 0.9 kb and 0.6 kb kb fragments appeared. As shown in Fig. 3, the 1.7 kb frament hybridized with LT-B, and the 0.9 kb fragment reacted with both LT-A and LT-B. It appears that

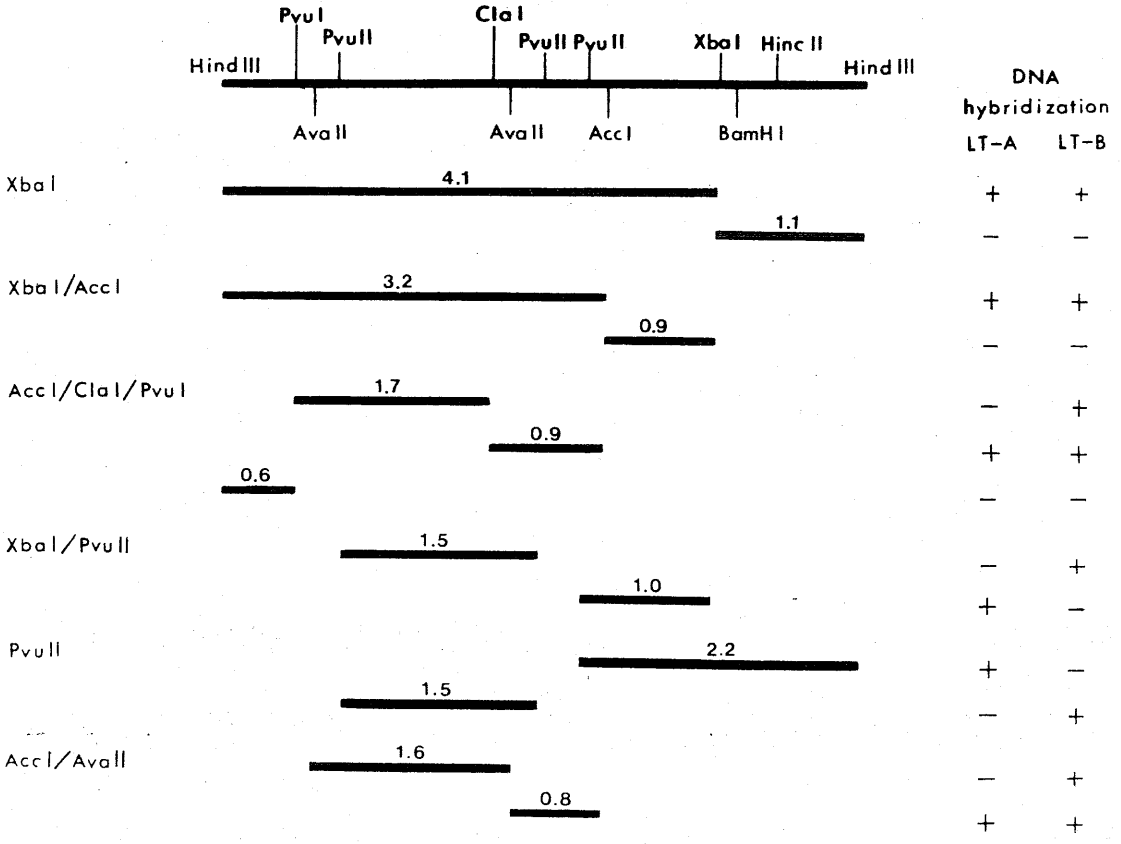

Fig. 3. Restriction map of pST2252 showing the approximate location of gene encoding of the LT-like toxin of *S. typhimurium*. Restriction enzymes used to digest the pST2252 and DNA fragments hybridized with A and B subunits of LT are illustrated below the restriction map. The symbols + and − indicate the positive and negative results of DNA hybridization, respectively. Numbers represent size of regions in kilobases.

the sequences which are homologous to the LT-B subunit gene are located on the 1.7 kb fragment. However, the 0.9 kb fragment contained a portion of the LT-B gene in addition to LT-A gene. It became clear that *S. typhimurium* LT-like toxin gene is located between the *Pvu* I and *Acc* I sites. Other double digestions of restriction enzymes confirmed this result. As indicated in Fig. 3, 1.5 kb and 1.0 kb *Xba* I-*Pvu* II fragments hybridized with LT-B and LT-A subunit genes, respectively. Similar results were observed after double digestion with *Acc* I and *Ava* II.

Cloning of LT-like toxin gene

The 5.2 kb *Hind* III fragment of *S. typhimurium* strain 22 was cloned onto *E. coli* plasmid pUC 19, producing a recombinant plasmid pST 2252 (Yang and Tan, 1989). Because the inserted DNA in this plasmid constituted a large portion of the LT-like gene, we constructed another hybrid plasmid consisting of a smaller insert at a different vector—pBluescript II KS (+). A 2.5 kb *Acc* I-*Pvu* I fragment of pST2252, containing both LT-A and LT-B genes, was ligated into *EcoR* V-cut pBluescript II KS (+) to produce plasmid pST2501 (Fig. 4A). In an

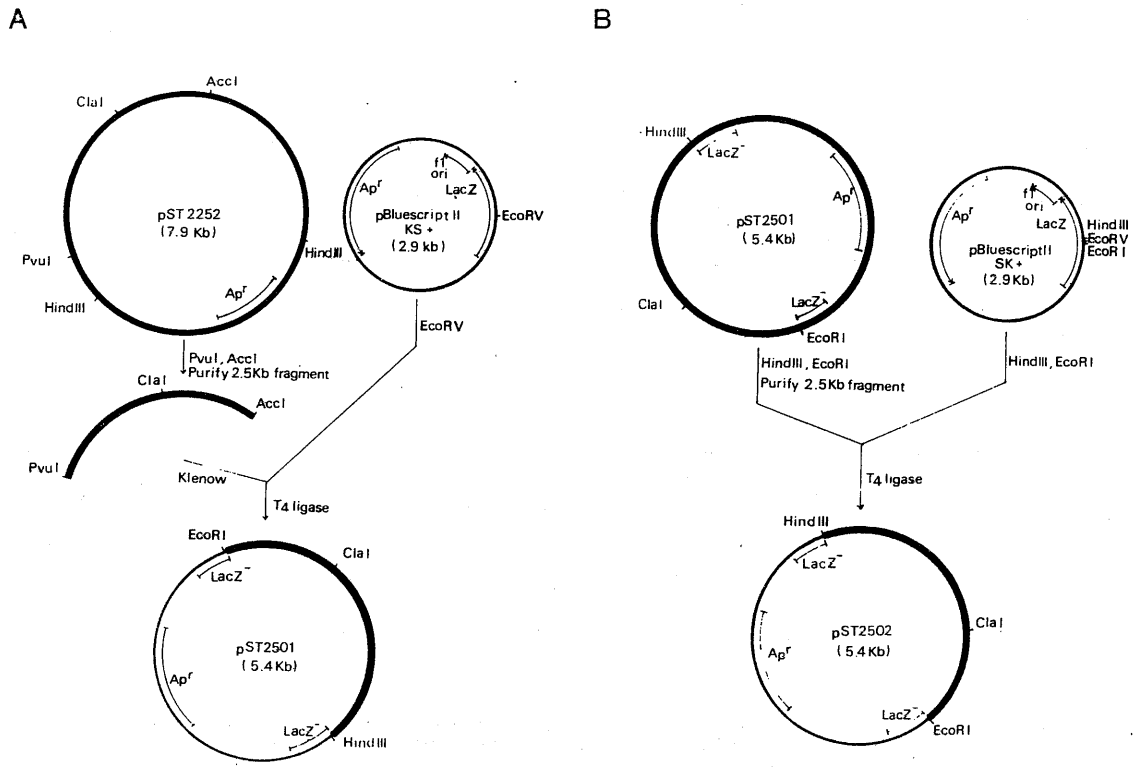

Fig. 4. Strategy for cloning the *Salmonella* LT-like toxin gene in *E. coli*. A 2.5 kb *Puv* I-*Acc* I fragment containing the entire enterotoxin gene was ligated to the *EcoR* V site of plasmid pBluescript II KS (+), forming a recombinant plasmid pST2501 (A) Similarly, the fragment carrying the LT-like toxin gene was isolated from pST2501 and ligated to the *EcoR* I-*Hind* III site of pBluescript II SK (+) to form pST2502 (B) Both plasmid constructs were tested for the expression of enterotoxin.

attempt to alter the orientation of this insertion, pST2501 was cleaved at the *EcoR* I and *Hind* III sites in the polylinker region, then ligated between the homologous sites of digested pBluescript II SK (+) to produce pST2502 (Fig. 4B). The ligated DNA mixture was used to transform competent cells of *E. coli* DH5α. Ampicillin-resistant *lac* Z clones were selected by colony hybridization with LT-A and LT-B as probes.

Enterotoxic activity encoded by recombinant plasmids

Enterotoxic activity produced by cloned recombinant plasmids was examined by a rabbit ileal loop assay and a skin permeability test. The results of these experiments are shown in Table 1; they demonstrated that crude extracts of *E. coli* (pST2252) increased vascular permeability in intracutaneous tests in rabbits causing fluid accumulation in the ligated ileal segments of adult rabbits. We found a strong correlation between the intestinal loop and permeability results with enterotoxin-producing *S. typhimurium* strain 22. Whole cell extracts of *E. coli* HB101, *E. coli* JM109, and *S. typhimurium* strain 2 were devoid of enterotoxic activity (Table 1). Two subclones of pST2252 were tested for toxic activity; results indicated that the *E. coli* DH5α carrying the recombinant

Table I
Detection of *S. typhimurium* enterotoxin by rabbit ileal loop assay and skin permeability test

Cell lysates of strains	Rabbit ileal loop assay Fluid secretion/loop length[a] (ml) (cm)	Skin induration (mm in diameter)
Escherichia coli[b]		
JM109 (pST22)	ND	20[d]
JM109 (pST2252)	1.29±0.19[c]	20
DH5α (pST2501)	0.38±0.04	ND
DH5α (pST2502)	1.63±0.03	ND
HB101 (pEWD299)	1.90±0.01	22
HB101	0.50±0.10	14
JM109	ND	12
Salmonella typhimurium[b]		
#22	1.37±0.13	20
# 2	0.30±0.10	0
PD buffer	0	0
TSB medium	0	0

a. Values are mean±standard deviation derived from three experiments. Control tests were inoculated with phosphate dilutent (PD) and Tryptic Soy Broth (TSB) medium.
b. Crude extracts were used in the rabbit ileal loop assay and in the skin permeability test. All data are the mean ratios of three experiments. ND: not determined.
c. Loops were considered positive with values above 1.0.
d. Diameter of induration at 24 hours.

plasmid pST2502 produced more toxin than (*E. coli* DH5α carrying) pST2252. However, the cell lysate strain containing pST2501 produced the least toxic activity in the toxin-intestinal assay. The cell extract of *E. coli* HB101 (pEWD299) containing the *E. coli* LT gene also showed high specific activity in both systems (Table 1).

Identification of recombinant plasmid-encoded proteins

To identify the products encoded by the cloned enterotoxin gene, plasmid pST2252 was transformed into the *E. coli* Eco867 minicell producing strain. Two specific polypeptides (26 kD and 15 kD) were detected by autoradiography following SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 5). Two proteins of 26 kD and 15 kD were also encoded by pST2501 (Fig. 5, lane b) and pST2502 (Fig. 5, lane c). These bands were not produced by *E. coli* Eco867 (pUC19 and pBluescript II KS (+)) (Fig. 5, lanes d and e). These results indicate that proteins are expressed irrespective of the orientation of insertion in the recombinant plasmids. However, biologically-active enterotoxin was only synthesized in pST2502. This suggests that the LT-like enterotoxin of *S. typhimurium* was encoded by the 2.5 kb *Pvu* I-*Acc* I fragment of pST2252. For comparison, we also analyzed the protein synthesized from LT plasmid pEWD299. Two distinct proteins with apparent molecular weights of 25.5 kD and 11.5 kD were detected by autoradiography (Fig. 5, lane f), corresponding to subunit A and subunit B, respectively. We suggest that the *Salmonella* enterotoxin was composed of a 26 kD subunit and a 15 kD subunit—a structure similar to the *E. coli* LT.

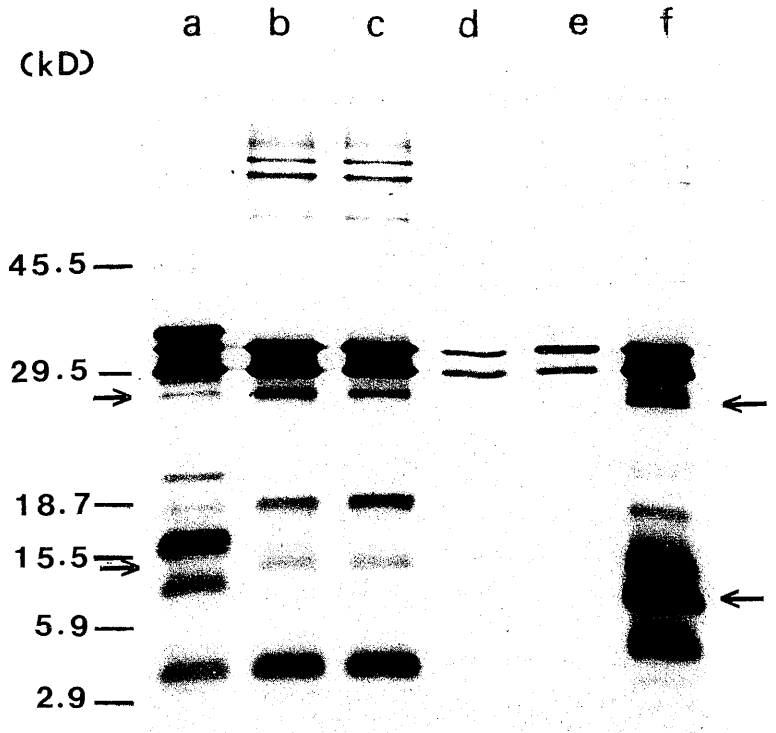

Fig. 5. Protein synthesis in *E. coli* minicells containing recombinant plasmids. Proteins were synthesized and radiolabeled wih [^{35}S] methionine as described in Materials and Methods, then resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (15% [wt/v] polyacrylamide). Lane a, pST2252 b, pST2501; c, pST2502; d, pUC19; e, pBluescript II SK (+); f, pEWD299. The migration of molecular weight markers is indicated in kilodaltons (kD) on the left. The arrows indicate the 25 kD, 11.5 kD, 26 kD, and 15 kD proteins, respectively.

DISCUSSION

Salmonella typhimurium strains have been found to produce a heat-labile enterotoxin which causes a secretory response in rabbit ileal loops and is neutralized by antiserum against LT produced by *E. coli*. Because of its similarities with biological activities of LT, we labelled the *Salmonella* enterotoxin *LT-like toxin*. Using the LT gene as a probe, we cloned the genes of the LT-like toxin from the plasmid of *S. typhimurium* strain 22. Through subcloning, minicell analysis, and biological assay, the enterotoxin gene was approximately localized at a DNA region of the 2.5 kilobase pair *Acc* I-*Pvu* II fragment. Based on hybridization reactions with both the A and B subunits of LT, we propose that there are regions of conserved nucleotide sequences between the *Salmonella* enterotoxin and LT. The extent of homology has not yet been determined but is under investigation. However, two proteins of molecular mass 26 kD and 15 kD were detected by SDS-PAGE; it has been reported that LT is composed of five 11.5 kD proteins and one 25.5 kD protein (Yamamoto *et al.*, 1984). We suggest that the organization of the *Salmonella* enterotoxin operon may be similar to the organization of the LT and CT operons.

Chopra *et al.* (1987) reported the cloning of cholera-like enterotoxin gene of *S. typhimurium*; indicated that the *Salmonella* enterotoxin gene was located on chromosome. They cloned a 6.3-kilobase

EcoR I-*Pst* I fragment of chromosomal DNA from the plasmidless strain Q1, and three subunits with molecular sizes of 45, 26, and 12 kilodaltons were expressed in minicells. An evolutionary relationship between the enterotoxin genes of *S. typhimurium* and *Vibrio cholerae* was therefore suggested. However, we preeviously demonstrated that the ability of *S. typhimurium* to produce enterotoxin was associated with the presence of a plasmid (Yang and Tan, 1989); strains lacking the plasmid were shown to be non-toxic. Recombinant plasmid containing the enterotoxin gene was able to restore full toxic activity when introduced into the plasmidless strain. This result demonstrated that the *Salmonella* enterotoxin gene, similar to that of LT, was located on a plasmid but not on a chromosome (Gyles *et al.*, 1974; Neill *et al.*, 1983). It is clear that this is evidence for the genetic heterogeneity of enterotoxin genes from individual isolates of *S. typhimurium*. We propose that the enterotoxin gene is part of a mobile element, or is carried by a very large transposable element. This assumption awaits protein chemistry studies of purified *Salmonella* enterotoxin for confirmation.

We recently found that the size of toxin-associated plasmids differed from strain to strain of *S. typhimurium* isolated in Taiwan. Notable differences were also noted in their hybridization reactions and restriction fragment patterns. A 5.2-kilobase *Hind* III fragment of *S. typhimurium* strain 22 composed of coding sequences of enterotoxin was used to study the organization of the gene in detail. The restriction map of the corresponding region in our study was quite different from the map of *Salmonella* enterotoxin genes reported by Chopra *et al.* (1987). We found that the enterotoxin gene was located within a 2.5 kb *Acc* I-*Pvu* I fragment, with no restriction sites for the *EcoR* I, *Hind* III, and *BamH* I enzymes. A detailed comparison of the two maps demonstrated the existence of restriction site heterogeneity within the structural gene of the *Salmonella* enterotoxin.

Recombinant plasmids containing the coding region of functional enterotoxin was isolated and measured by rabbit ileal loop assay. Although the availability of the cloned *Salmonella* enterotoxin gene allowed us to study the molecular structure of the toxin gene, the size of the gene is not yet certain. Insertional mutagenesis or *in vitro* deletion techniques are needed to localize the coding sequence of functional enterotoxin. In addition, the nucleotide sequence of the toxin gene needs to be determined so that the amino acid sequence of the toxin can be deduced. The primary structure of the toxin can then be analyzed and the possible function of the molecule formulated. Furthermore, a comparison between the *Salmonella* enterotoxin and CT or LT can be made so that differences and similarities in molecular structure can be discerned. The information will help us speculate on the toxin's mode of action.

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鼠傷寒桿菌腸毒素基因之鑑定與限制酶分析

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由腹瀉病人分離出可產生腸毒素之鼠傷寒桿菌 (*Salmonella typhimurium*)，利用 *E. coli* 之 LT 基因探針測出毒素基因位於質體之 5.2 kb 之 *Hind* III 片段內，帶此 DNA 片段之重組質體 pST2252 可爲多種限制酶作用，其中 *Xba* I, *BamH* I, *Hinc* II, *Pvu* I, *Cla* I, *Ava* II, *Pvu* II, *Sac* I 與 *Acc I* 等九種限制酶之作用位置皆已訂出，由南氏吸漬法證明毒素基因位於 *Acc* I 至 *Pvu* I 間之 2.5 kb 範圍內。將 *Acc* I 至 *Pvu* I 間之 2.5 kb DNA 片段，分別插入質體 pBluescript II KS(+) 與 SK(+) 形成兩重組質體 pST2501 與 pST2502，以 *E. coli* minicell 分析，可產生分子量爲 26 kD 與 15 kD 之兩種蛋白質，其中含 pST2502 之細胞抽取物可造成白兎廻腸及皮膚明顯的腫脹反應，故知毒素基因已被完整地選殖出來，其產生之腸毒素與腹瀉有直接的關係。