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Development of Verotoxin 2- and Verotoxin 2 Variant (VT2v)-Specific Oligonucleotide Probes on the Basis of the Nucleotide Sequence of the B Cistron of VT2v from *Escherichia coli* E32511 and B2F1

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We and others have noted that there are serological differences between verotoxin 2 (VT2) (also known as Shiga-like toxin II) produced by *Escherichia coli* C600(933W) and the VT2 variant (VT2v) produced by strain E32511. Recent reports have described nucleotide sequence differences between the VT2v B subunit cistron of E32511 and B2F1 and that of VT2. We have confirmed the sequence differences and have used them to design oligonucleotide probes which differentiate the B subunit cistron of VT2v from that of VT2. Isolates of VT-producing *E. coli* obtained from human as well as food and veterinary sources were classified according to the toxin phenotype by using a toxin neutralization assay with VT2-specific monoclonal antibody and VT2v-specific polyclonal antisera. Using the oligonucleotide probes in colony hybridization, we detected 35 of 35 VT2 producers and 16 of 16 VT2v producers. One VT2 producer was falsely identified as containing the VT2v gene. The E32511 strain in our collection hybridized only with the VT2-specific probe. Southern hybridization of radiolabeled oligonucleotide probes showed that strains carried zero to one copy of the VT2 gene and zero to two copies of the VT2v gene. We conclude that colony hybridization with the VT2- and VT2v-specific probes is highly predictive of the toxin phenotypes in the clinical isolates described in this study.

The verotoxin (VT) (*Escherichia coli* Shiga-like toxin [SLT]) family is composed of two main groups (13). One typified by VT1 (SLT-I) is almost identical to the Shiga toxin of *Shigella dysenteriae* 1 and is neutralized by antibodies to Shiga toxin (13, 16, 22). The prototypes of the second group are VT2 and SLT-II specified by the bacteriophages ϕ 32511 and ϕ 933W, respectively (4, 5, 13, 21, 22). It has become apparent that there are several variants of VT2 (SLT-II). The toxin associated with edema disease of swine is partially neutralized by VT2 (SLT-II) antiserum and has been designated VTe (3, 23), SLT-IIv (7, 25), and SLT-IIVp (19). It is associated with porcine disease. Furthermore, it binds primarily to the receptor globotetraosyl ceramide (3, 19), while the receptor for VT2 (SLT-II) is globotriosyl ceramide (19).

Head et al. (9) reported that a toxin purified from *E. coli* E32511 was only partially neutralized by monoclonal antibody BC5BB12, which recognizes the B subunit of VT2 (SLT-II) (5). Head et al. (9, 10) designated this toxin VT2. This toxin also binds to globotriosyl ceramide (24). Subsequently, the same toxin was designated SLT-IIc (20) and the VT2 variant (VT2v) (23). Oku et al. (17) found that the VT produced by strain B2F1 cross-reacted with VT2 (SLT-II) (17). Ito et al. (11) reported that this strain contains two VT operons, designated vtx2ha and vtx2hb. They called the toxin VT2vh (11), while Samuel et al. (19) named it SLT-IIvh (19). Since the nomenclature in the field is becoming extremely confused, Tyler et al. (23) suggested that henceforth SLT-II be named VT2, swine edema disease toxin be named VTe, and the variant of VT2 previously designated VT2 (9, 10), VT2vh (11, 17), and SLT-IIc (20) be named VT2v. We use that system in this report.

The original purpose of this study was to determine the basis for the serological difference between VT2 produced by *E. coli* C600 lysogenized by ϕ 933W and the VT2v purified by Head et al. (9) from strain E32511 by nucleotide sequence analysis of the B cistron of their respective VT operons. We were also interested in comparing the nucleotide sequences of the B cistrons of strains B2F1 and E32511, since preliminary studies indicated that the two toxins had similar neutralization characteristics. The nucleotide sequence differences in the B cistrons were then used to develop oligonucleotide probes which were highly predictive of the toxin phenotype (immunotype) determined by a neutralization test. An important finding was that the strain of E32511 used by Head et al. (10) for toxin production does not carry the VT2 gene and carries the gene specifying VT2v only.

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MATERIALS AND METHODS

Bacterial strains and media. Human disease-associated isolates of verotoxigenic *E. coli* and negative control strains,

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including an enterotoxigenic *E. coli* strain (strain H10407), an enteropathogenic *E. coli* strain (strain E2348), and an enteroinvasive *E. coli* strain (strain CL114), were from the collection of M. A. Karmali (2). Animal and food isolates were from the collection of R. Clarke. Strains were serotyped courtesy of H. Lior, Laboratory Center for Disease Control, Ottawa, Ontario, Canada. The serotype and toxin phenotype (determined as described below) are given below (see Table 4 and the footnotes to Table 4). Strains were grown in L broth (14) or on L agar.

Hybridization studies and Southern blot analysis. The nucleotide sequences of the oligonucleotide probes (see Table 1) were based on the nucleotide sequences for the B-subunit-coding region of the SLT-II (VT2) gene published by Jackson et al. (12) and on the sequence of the B subunit cistron of VT2v determined in this study (see Fig. 1). Oligonucleotides were synthesized by the automated phosphoramidite coupling method on an Applied Biosystems synthesizer. Oligonucleotides were 5′-end-labeled with [γ-^{32}P]ATP (Amersham, Oakville, Ontario, Canada) and T4 polynucleotide kinase (Boehringer Mannheim, Laval, Quebec, Canada) and were purified on NENsorb columns according to the instructions of the manufacturer (Dupont, Boston, Mass.). The specific activity of these oligonucleotide probes was 2×10^6 to 4×10^6 cpm/pmol.

For colony blots, strains were picked and inoculated on nitrocellulose membranes (Millipore, Bedford, Mass.), which were laid onto L agar. After overnight growth, the filters were lifted and placed colony side up onto blotting paper saturated with 0.5 M NaOH for 5 min. They were sequentially transferred onto blotting paper soaked with 1 M Tris-HCl (pH 7.4) twice for 5 min each time and, lastly, onto blotting paper soaked with 0.5 M Tris-HCl (pH 7.4)–1.5 M NaCl for 5 min. Filters were air dried and were then baked at 80°C for 15 min (15). Filters were prehybridized in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt's solution–0.2% sodium dodecyl sulfate (SDS) for 30 min at 20°C and were then washed in 6× SSC. Hybridization with probe (2×10^6 cpm/ml) was carried out at 20°C for 1 h in hybridization buffer containing 50 μg of sheared denatured salmon sperm DNA per ml, 6× SSC, 10× Denhardt's solution, and 0.2% SDS. Filters were washed in 6× SSC buffer containing 0.2% SDS at room temperature and were then washed at various temperatures and exposed to x-ray film between the washing steps.

For Southern blot analysis, total bacterial DNA prepared by the cetyl-tetraethyl ammonium bromide procedure (26) was digested with *Eco*RI and subjected to electrophoresis through a 0.9% agarose gel. *Eco*RI fragments were transferred bidirectionally to GeneScreen Plus membranes (Dupont) and were probed with the cistron-specific probe (ES135 or AB157). After washing in 6× SSC at 55°C, the blots were subjected to autoradiography.

Preparation of single-stranded DNA for sequencing analysis. An asymmetric polymerase chain reaction was used to generate a single-stranded DNA template for nucleotide sequence analysis. A 100-μl reaction containing primer ratios of 50:0.5 pmol generated single-stranded DNA of either polarity after a 40-cycle run (8). Primers complementary to the 5′- and 3′-flanking regions of the B subunit cistron of SLT-II were P1 (1A2) (GTCACAGTTTTTATATAC), representing nucleotides 1165 to 1182 of the sequence of Jackson et al. (12) for plus strand synthesis, and P2 (2141) (AGATTACACTTGTTACC), which was complementary to nucleotides 1502 to 1518 of the published sequence for minus strand synthesis, starting 30 bases downstream from the termination codon of the B cistron. One microgram of chromosomal DNA or 10^5 CFU of boiled bacteria was used as the target. DNA was extracted from strains C600 (933W), E32511, and B2F1. Reactions were run to a maximum of 40 to 45 cycles in final concentrations of 2 mM Mg^{2+} and 200 μM precursor deoxynucleoside triphosphates. Single-stranded DNA was isolated on NENsorb columns, dried in vacuo, and resuspended in distilled water. Sequencing of both strands was done by the Sanger method by using *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) at 70°C (8).

Antisera. Polyclonal rabbit antisera were obtained by immunizing rabbits with purified toxins. VT1 was purified from *E. coli* H30 (18), while VT2v was purified from strain E32511 as described by Head et al. (10). VT2 antiserum was prepared by using toxin preparations obtained from strain NM522(pEB-1) (5). The monoclonal antibody BC5BB12, which recognizes the B subunit of VT2 (5), was also used and was the gift of N. Strockbine, Centers for Disease Control, Atlanta, Ga.

Determination of the toxin phenotype. To obtain crude toxin preparations, bacterial strains were grown overnight in brain heart infusion broth, and the culture supernatants were filter sterilized. For the neutralization assays, constant amounts of antiserum were added to various amounts of supernatant containing toxin(s). The culture supernatants were fivefold serially diluted and were mixed with 50 μl of antiserum. Sufficient antiserum was used to completely neutralize the homologous toxin produced by all strains in this study. Serially diluted toxin preparations mixed with various combinations of antisera to VT1, VT2, and VT2v were applied to Vero cell monolayers, and the cells were then incubated for 72 h. Preimmune serum mixed with serially diluted supernatant was used as a control. The monolayers were fixed with 2% formalin and were stained with crystal violet before being examined for adherent cells by light microscopy. Significant neutralization was defined as at least a 25-fold reduction in cytotoxin titer compared with that in controls consisting of toxin incubated with preimmune serum. Complete neutralization consisted of an absence of a cytotoxic effect in all wells. Strains that produced cytotoxic activity, that were completely neutralized by anti-VT2v (E32511) antiserum, and that were only partially neutralized by anti-VT2 antiserum (BC5BB12) were considered to produce VT2v. Those strains that produced toxins and that were neutralized equally well by anti-VT2 and anti-VT2v antiserum were considered to produce VT2. The procedure will be described in more detail elsewhere (14a). The VT1 phenotype for these strains was determined previously (2, 2a).

For the type strains (see Table 3), neutralization assays were also performed by the method of Gannon et al. (6). Culture supernatants were used as a source of toxin from the various strains. Ten 50% cytotoxic dose (CD_{50}) units were mixed with dilutions of antisera and were applied to Vero cell monolayers. After incubation for 72 h, monolayers were fixed, stained with crystal violet, and examined by light microscopy. Assays were done in triplicate. The endpoint titer of each assay was determined as the reciprocal of the highest dilution of neutralizing antiserum which resulted in 50% detachment of the cell monolayer when it was incubated with 10 CD_{50} units of toxin.

Nucleotide sequence accession number. The nucleotide sequence data reported here appear in the GenBank nucleotide sequence data base under the accession number M76738.

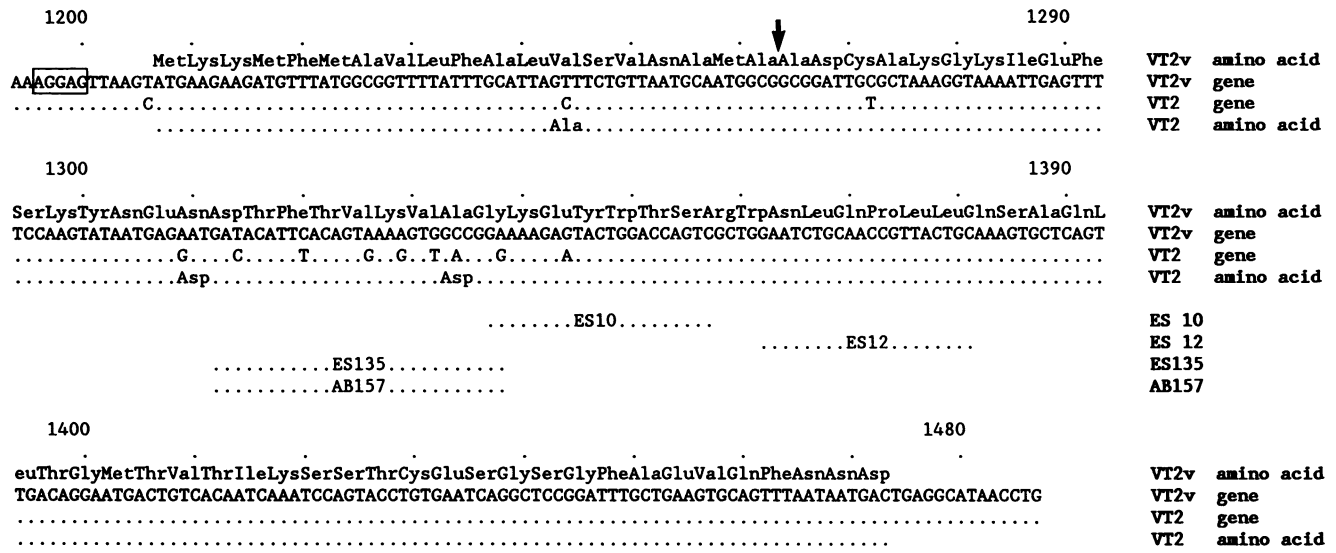

FIG. 1. Nucleotide sequence of the B subunit cistron of the VT2v operon of strains E32511 and B2F1. The nucleotide sequence obtained and shown here is identical to the nucleotide sequence of Vtx2ha of strain B2F1 published by Ito et al. (11) and is compared and numbered according to the VT2 (SLT-II) sequence from nucleotides 1194 to 1487 of Jackson et al. (12). Nucleotides that differ in the VT2 (SLT-II) sequences are shown below the VT2v sequences, while amino acid residues which differ are shown on the lowest line. The putative signal peptidase cleavage site is indicated by the arrow. The potential Shine-Dalgarno sequence for VT2 (SLT-II) and VT2v is boxed. The nucleotide positions of the synthetic oligonucleotide probes are indicated. The VT2v-specific primer described by Tyler et al. (23) includes nucleotides 1316 to 1335.

RESULTS

Nucleotide sequence analysis. The nucleotide sequence of both strands of a 354-bp region that contains the B cistron and that is encompassed by the primers 1A2 and 2141 is presented in Fig. 1. The nucleotide sequence was identical in both strains E32511 and B2F1 and was subsequently found to be identical to that of the vtx2ha B cistron of B2F1 recently reported by Ito et al. (11). Recently, Schmitt et al. (20) have obtained the same sequence for the SLT11-c B cistron from strain E32511. There were 11 nucleotide differences between this sequence and that of the VT2 (SLT-II) B cistron of bacteriophage 933W, which was determined in this study as a control and had been previously reported by Jackson et al. (12). Seven of these differences were in the region spanned by the probe ES135 (Fig. 1). Only three amino acid changes were predicted. One was in the putative signal peptide. The two amino acid changes were in the mature B subunit polypeptide. VT2 Asp-16 was changed to Asn and VT2 Asp-24 was changed to Ala. These changes resulted in a net increase of two in the charge of the B-subunit monomer and are consistent with the increased pI noted for VT2v compared with that noted for VT2 (SLT-II) (4, 5, 10, 17).

Specificities of the VT2 and VT2v probes. Probes ES10 and ES12 were taken from regions which are conserved in the VT2 and VT2v sequences, while ES135 was taken from the region which differs (Table 1; Fig. 1). On the basis of the nucleotide sequence of the B cistron of VT2v found in strains B2F1 and E32511, we synthesized a VT2v-specific probe designated AB157 which corresponds to ES135 in nucleotide position but which contains the VT2v sequence. The four oligonucleotide probes listed in Table 1 were initially tested for specificity against a limited number of strains in colony hybridization assays.

Blots were washed first at 50°C and then at 55°C. The results (Table 2) show that AB157 bound only to strains E32511 and B2F1, which produced VT2v, but not to the VT2-producing strain *E. coli* C600(933W). In contrast, ES135 hybridized only with the VT2 producer C600(933W) and did not hybridize with E32511 or B2F1. This suggests that probe ES135 is specific for VT2 producers, while AB157 is specific for VT2v producers. Probe ES10, which is specific for VT2, bound to B2F1 DNA at both 50 and 55°C, while it bound to E32511 DNA at 50°C but not at 55°C. These results are consistent with the presence of the vtx2hb B cistron in B2F1. This gene produces a B subunit with the same amino

TABLE 1. Sequences of synthetic oligonucleotides tested as probes for genes of VT2 and VT2v

Oligonucleotide	Polarity[a]	Nucleotide base sequence	Postion no.
ES10[b]	+	GGA AAG AAT ACT GGA CCA GTC	1337–1357
ES12[b]	−	GCA GTA ACG GTT GCA GAT TC	1362–1381
ES135[b]	−	CCC GTC AAC CTT CAC TGT AAA TGT GTC	1312–1338
AB157[c]	+	GAT ACA TTC ACA GTA AAA GTG GCC GGA	1312–1338

[a] Oligonucleotides ES10 and AB157 have the sequence of the sense (+) strand, while oligonucleotides ES12 and ES135 have the sequence of the antisense (−) strand.

[b] The nucleotide sequences and positions for these oligonucleotides are based on the published nucleotide sequence of SLT-II (12).

[c] The nucleotide sequence of AB157 is based on the derived sequence given in Fig. 1, and its nucleotide positions correspond to the published data given by Jackson et al. (12).

TABLE 2. Colony blot hybridization with VT2- and VT2v-specific oligonucleotide probes

E. coli Culture (toxin)[a]	Hybridization with the following oligonucleotide probes[b]			
	ES10	ES12	ES135	AB157
C600 (933J) (VT1)	−	−	−	−
C600 (933W) (VT2)	+	+	+	−
E32511 (VT2v)	−[c]	+	−	+
B2F1 (VT2v)	+	+	−	+
412[d] (VT2e)	−	−	−	−
H10407 (enterotoxigenic)	−	−	−	−
E2348 (enteropathogenic)	−	−	−	−
CL114 (enteroinvasive)	−	−	−	−

[a] The strains were tested for cytotoxicity by toxin neutralization (see text).
[b] The oligonucleotide probes were eluted from the colony blot nitrocellulose membranes sequentially at temperatures of 50 and 55°C. The results of elution at 55°C are given here.
[c] Probe ES10 hybridized with strain E32511 at 50°C but not at 55°C.
[d] Strain 412 is a VTe-producing *E. coli* isolate associated with porcine edema disease (6).

acid sequence as that of the vtx2ha B cistron. However, the nucleotide sequence of vtx2hb is identical to the VT2 sequence at positions 1338 and 1344, which are both included in the ES10 probe (11). Of particular interest was the finding that the VT2-specific probe ES135 did not hybridize with the strain of E32511 obtained from the collection of M. A. Karmali. This is the same strain previously used by Head et al. to prepare VT2v toxin (9, 10). Taken together, these results show that this strain does not contain a copy of the VT2 (SLT-II) sequence.

Neutralization studies. Toxin neutralization studies were done to determine whether the toxins produced by strains E32511 and B2F1 differed from that of C600 (933W) in their antigenic nature. Toxin neutralization was tested by using homologous and heterologous antisera, and the neutralizing antibody titer of antisera was determined against 10 CD_{50} units of the various verotoxins (Table 3). When polyclonal anti-VT2 antibody was used for neutralization, the neutralization titer against VT2 was only five times that against VT2v. By using the VT2-specific monoclonal antibody BC5BB12, the titer against VT2 was 40 times that against VT2v preparations from strains E32511 and B2F1. Polyclonal antiserum raised against VT2v purified from E32511 neutralized both VT2 and VT2v at the same dilution. We noted that the titers for all neutralizations were identical for crude toxin prepared from strains E32511 and B2F1.

TABLE 3. Neutralization of VT preparations by homologous and heterologous antisera

Toxin source	Neutralizing antibody titer[a]				
	Anti-VT1	Anti-VT2	Anti-VT2 (BC5BB12)	Anti-VT2v	Anti-VTe
H30	3,125	<5	<100	<5	<5
933W	<5	625	32,000	625	25
E32511	<5	125	800	625	125
B2F1	<5	125	800	625	125
412	<5	125	100	625	3,125

[a] The titer is expressed as the reciprocal of the highest dilution of serum which resulted in 50% detachment of the cell monolayer when it was incubated with 10 CD_{50} units of toxin preparation. The series of dilutions used to neutralize toxin preparations were fivefold for polyclonal antisera and twofold starting from an initial 1:1,000 dilution of the monoclonal antibody BC5BB12 when it was tested against toxin from strain C600 (933W) or a 1:100 dilution when it was tested against the other toxin sources. Titers against homologous VTs are underlined.

TABLE 4. Determination of toxin genotype by specific oligonucleotide probes

Toxin phenotype	No. of strains	No. (%) of strains that hybridized			
		ES12	ES135	AB157	ES135-AB157
VT2[a]	12	12 (100)	11 (92)	0	1 (8)
VT1 and VT2[b]	23	23 (100)	23 (100)	0	0
VT2v[c]	14	14 (100)	0	10 71	4 (29)
VT1 and VT2v[d]	2	2 (100)	0	2 (100)	0
Total	51	51 (100)	34 (67)	12 (24)	5 (9)

[a] Serotypes, numbers of strains are as follows: O157:H7, 6; O57:H⁻, 1; O118:H30, 1; O?, 3; O6:H34, 1.
[b] Serotypes, numbers of strains are as follows: O157:H7, 17; O157:NM, 3; O111:H8, 1; O111:NM, 2.
[c] Serotypes, numbers of strains are as follows: O157:H⁻ (E32511), 1; O117:H4, 2; O113:H21, 4; O91:H21 (B2F1), 1; O22:H16, 1; O2:H29, 1; O?:H119, 2; O?H21, 1; O?:H2, 1.
[d] Serotypes, numbers of strains are as follows: O145:NM, 1: O?:H21, 1.

Use of VT2 and VT2v probes for clinical strains. Fifty-one strains were available from the collections of M. A. Karmali and R. Clarke. The toxin phenotypes (VT2 or VT2v) of these strains were determined by the neutralization assay described in the Materials and Methods. This method detected strains which produce VT2v alone or in combination with VT2. However, because no antiserum which neutralized VT2v without neutralizing VT2 was available, by this procedure it was impossible to know whether VT2 was produced in addition to VT2v. The strains were examined with probes ES135 and AB157 by using colony hybridization (Table 4). All thirty-five strains that produced VT2 were correctly identified with ES135 as having the VT2 genotype. One O157 strain also hybridized with the VT2v probe AB157. This was taken to be a false-positive result. All 16 strains with the VT2v phenotype were identified by the VT2v probe AB157. Four of these strains also hybridized with ES135. In this study none of the O157:H7 strains had either the VT2v genotype or the VT2v phenotype. All four of the O113:H21 isolates had both VT2v and VT2 genotypes and the VT2v phenotype.

Southern hybridization studies. To determine whether multiple toxin gene copies were carried in the genomic DNAs of strains E32511 and B2F1 and three O113:H21 strains, Southern blot analysis was performed. Figure 2 shows the hybridization of blots of genomic DNA digested with *Eco*RI and hybridized with the VT2-specific probe ES135 and the VT2v-specific probe AB157. It can be seen that strain B2F1 (lane 7) carries two copies of the VT2v gene, while E32511 carries only one copy of the VT2v gene and no VT2 gene. The three strains of serotype O113:H21 carry one copy of the VT2 gene and one to two copies of the VT2v gene. In the case of lane 5, both probes may have bound to the same fragment, and it is not clear whether there were two genes on fragments of the same size or whether a single sequence bound to both probes.

DISCUSSION

The results presented in this report show that the E32511 strain in the collection of M. A. Karmali carries only a single copy of the VT2v operon. This result clearly differs from the result of the recent study of Schmitt et al. (20), who reported

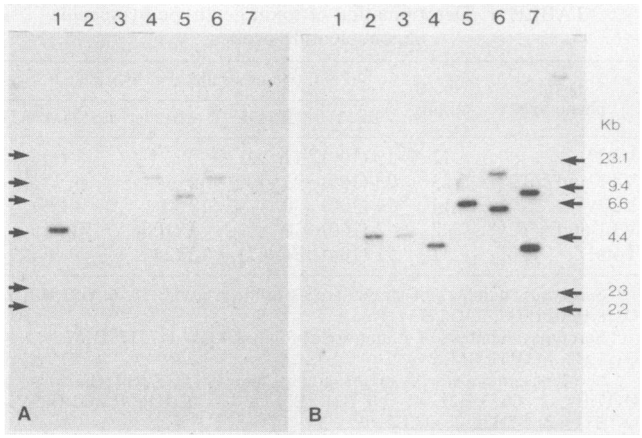

FIG. 2. Gene copies of SLTII and VT2 in clinical isolates. Total bacterial DNAs were digested with *Eco*RI and hybridized with oligonucleotide probe ES135 (A) or AB157 (B) by Southern blot analysis. Lane 1, CL3 (O?); lanes 2 and 3, E32511 (O157:H$^-$); lane 4, RC490 (O113:H21); lane 5, CL3A2 (O113:H21); lane 6, RC3 (O113:H21); lane 7, B2F1 (O91:H21). The numbers to the right of the arrows are marker DNA fragments (in kilobase pairs).

that E32511 contains both the VT2 and the VT2v operons. The strain used in the present study was stored on agar and likely represents a derivative of the original strain E32511, which has been spontaneously cured of the prophage E32511. Therefore, the VT2v purified by Head et al. (10) was produced from a strain with only a single toxin operon and must be homogeneous. The genotype of this strain was confirmed by Southern blot analysis; this should allay the concerns expressed by Schmitt et al. (20) regarding the purity of this toxin and the antisera raised against it. The sequence of the VT2v B cistron presented here and elsewhere (11, 20) predicts a significantly higher pI of the mature B subunit since two aspartic acid residues are substituted with neutral residues. Head et al. (10) used a chromatofocusing step in purification. The pI of 6.5 they reported for VT2v is significantly higher than the pI of 5.0 to 5.2 reported for VT2 by Dickie et al. (4) and Downes et al. (5). Therefore, the purification procedure used (10) should yield pure VT2v toxin, even in strains that produce both VT2 and VT2v.

The original aim of this study was to determine the basis for the difference in the neutralization titer of monoclonal antibody BC5BB12 against VT2 and VT2v. Since this monoclonal antibody is specific for the B subunit, we determined the nucleotide sequence of this cistron. The results confirm those obtained by Ito et al. (11) and Schmitt et al. (20), which were published recently. The two amino acid substitutions presumably account for the difference in neutralization titers. It is worth noting that the sequences of the B cistron of the VT2v operon of E32511 and of the vt2vha and vt2vhb operons of B2F1 all predict the same amino acid sequence for the mature B subunit polypeptide (11, 20). This is reflected by the identical data we obtained for the neutralization of toxins produced by B2F1 and our E32511 strain.

We produced probes ES135 and AB157, which are specific for the VT2 and VT2v sequences, respectively. These probes were taken from a region where there are seven nucleotide differences between the two B cistrons. It should be noted that only one of these results in an amino acid substitution in the B polypeptide. Therefore, it is possible that the probe could bind to a sequence which does not encode the amino acid substitution. This could have accounted for the single apparently false-positive VT2v genotype obtained with the AB157 probe. The basis for this needs to be investigated further, since it is possible that this strain contains a sequence which is similar to AB157 but which does not encode the amino acid substitution. However, other than the result obtained with this single strain, there was a complete association between the VT2 and VT2v phenotypes and hybridization with probes ES135 and AB157, respectively. Use of the probe is more convenient than the neutralization test. In addition, the neutralization test cannot detect strains which produce both VT2 and VT2v. Our results do suggest, however, that the neutralization test is a valid way of identifying VT2v-producing strains. It appears to be much more useful than tests based on differential cytotoxicity on various cell lines, such as Vero and HeLa cells (23). We found that the VT2v gene occurred in all O113:H21 isolates available for testing. We did not find VT2v production in any of our O157:H7 isolates. In contrast, Tyler et al. (23) reported that a significant number of O157:H7 isolates have the VT2v genotype.

Tyler et al. (23) have recently reported polymerase chain reaction primers which also differentiate the B cistron of VT2 from that of VT2v. The primer for the plus strand of the VT2v primer set was taken from the same region as our probe AB157, while the other primer was taken from a noncoding region downstream from the B cistron (23). Therefore, this procedure also may not detect the specific changes in nucleotide sequence which result in the antigenic differences between VT2 and VT2v. Tyler et al. (23) also described a protocol which would allow distinction between the vtx2ha and vtx2hb sequences (23). This distinction is of significance only for genotyping strains, since both sequences encode identical amino acid sequences in the mature B subunit polypeptide (11).

It is not clear whether the amino acid substitutions in the VT2v B subunit have significance with respect to the pathogenesis of the hemolytic uremic syndrome or hemorrhagic colitis (12). It is possible that VT2 and VT2v could have slightly different receptor specificities. Differences in cytotoxic activity on different cell lines have been documented (19). Moreover, it is possible that people who are immune to VT2 might not be immune to VT2v. This might be of significance if people immune to VTs were protected from hemolytic uremic syndrome. However, testing of pooled human immune serum globulin suggests that there may not be VT2-neutralizing antibodies in the general population (1).

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