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Expression of Staphylococcal Enterotoxin C_1 in *Escherichia coli*

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The structural gene encoding staphylococcal enterotoxin C_1 was cloned into *Escherichia coli* and localized on a 1.5-kilobase *Hin*dIII-*Cla*I DNA fragment by subcloning. The toxin was partially purified from *E. coli* clones and shown to be immunologically identical to enterotoxin C_1 from *Staphylococcus aureus*. The cloned toxin also had the same molecular weight (26,000) and charge heterogeneity as staphylococcus-derived enterotoxin. Toxins from both sources were equally biologically active.

Several exotoxins produced by *Staphylococcus aureus* and *Streptococcus pyogenes* share numerous biological and biochemical properties. This group of related toxins includes the staphylococcal enterotoxins (3), and pyrogenic exotoxins (33), toxic shock syndrome toxin 1 (TSST-1) (4), and streptococcal pyrogenic exotoxins (2). All of these toxins induce lymphocyte mitogenicity, immunosuppression, pyrogenicity, and enhancement of lethal endotoxin shock (12, 19, 29, 33, 37, 40). Staphylococcal enterotoxins are distinguished from other toxins in this group by their additional capacity to induce emesis and diarrhea in animals after oral administration (3, 14). Likewise, streptococcal pyrogenic exotoxins are unique in their capacity to induce heart damage (36).

These toxins are small proteins with reported molecular weights of less than 30,000. Staphylococcal enterotoxins have similar amino acid compositions (3), and the amino acid sequences of at least enterotoxins B and C_1 possess extensive homology (17, 35). In addition, Johnson et al. (18) demonstrated significant amino acid sequence homology between enterotoxin B and streptococcal pyrogenic exotoxin A based on nucleotide sequence analysis.

Enterotoxin A and streptococcal pyrogenic exotoxin A have been shown to be transferred by bacteriophage (7, 19). In contrast, the gene for TSST-1 is chromosomally located (22). The location of genes for other toxins in this group remains unclear.

We report in this paper the molecular cloning of the gene encoding enterotoxin C_1. The cloned toxin prepared from *Escherichia coli* is biologically active and has biochemical properties identical to those of staphylococcus-derived enterotoxin C_1.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* MN Don, a clinical isolate from blood of a patient with toxic shock syndrome, was used for production of enterotoxin C_1 and as a source of DNA for construction of a genomic library. This strain, submitted by P. F. Sparling, University of North Carolina, Chapel Hill, makes enterotoxin C_1 while not expressing any other enterotoxins, TSST-1, or exfoliative toxins. Identification of enterotoxin C_1 was confirmed by using reference antisera supplied by E. Schantz, University of Wisconsin, Madison, and by comparison of the N-terminal amino acid sequence with that previously reported for the toxin (15, 35). A total of 21 amino acids constituting the N terminus of the protein were analyzed. Although the data did not permit assignment of amino acids to positions 10 and 12, the remaining residues were in complete agreement with the previously reported sequence (35). *E. coli* K12 strains RR1 (F$^-$ *hsdS20 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44* λ^- [10]) and JM83 (*ara* Δ*lac-pro strA thi* φ80d*lacZ*ΔM15 [27]) were used for propagation of vector plasmids and as recipients of DNA in transformation procedures.

Antiserum preparation. Hyperimmune antiserum was prepared by immunization of American Dutch-belted rabbits (32) with purified enterotoxin C_1 from *S. aureus* MN Don.

Enzymes. *Bam*HI, *Bgl*II, *Acc*I, and calf intestinal alkaline phosphatase were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. *Cla*I, *Hin*dIII, *Mbo*I, and T4 DNA ligase were purchased from New England BioLabs, Inc., Beverly, Mass. Reactions involving these enzymes were performed as directed by the manufacturers.

Preparation of staphylococcal DNA. DNA was obtained from *S. aureus* by a modification of the method described by Macrina et al. (25) except that lysostaphin (Sigma Chemical Co., St. Louis, Mo.) (15 μg/ml) was used to promote cell lysis. A partial digest of the genome was prepared for cloning by incubating the DNA for 1 h with *Mbo*I (0.63 U/μg of DNA).

DNA purification. Plasmids were isolated from exponential-phase cultures grown in LB medium (5) by the procedure of Holmes and Quigley (16) after amplification with chloramphenicol (180 μg/ml) (13). The plasmids were further purified by hydroxyapatite chromatography followed by extraction with phenol-chloroform. Digested DNA for cloning and subcloning was purified by electrophoresis in agarose gel slabs (19). Nucleic acids were recovered from gels by trough electroelution (26) or the rapid-freezing method of Smith (38).

DNA ligation and transformation. Plasmids pBR328 (39) and pUC13 (41) were used as vectors in cloning and subcloning experiments, respectively. Vector and insert DNAs (25 to 100 ng) were mixed in a 1:1 (wt/wt) ratio. Ligations were performed overnight at 14°C with 200 U of T4 DNA ligase. Competent cells from cultures of *E. coli* RR1 or JM83, prepared by the method of Kushner (23), were used as recipients of DNA in transformation experiments.

Selection procedures. *E. coli* RR1 cells transformed with pBR328 recombinants were plated on LB agar containing ampicillin (100 μg/ml) and replica plated onto media contain-

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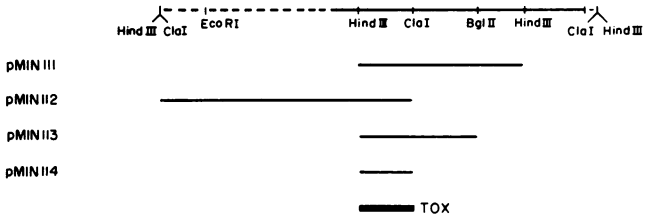

FIG. 1. Physical map of pMIN101 showing the 7.1-kb insert (solid line) from the partially digested genome of *S. aureus* MN Don and pBR328 vector (broken line). Restriction fragments containing *entC*$_1$ produced by digestion of pMIN101 with combinations of *Hin*dIII, *Cla*I, or *Bgl*II were subcloned into pUC13 to produce pMIN111-pMIN114.

ing tetracycline (12.5 μg/ml). *E. coli* JM83 cells transformed with pUC13 recombinants were plated on LB medium containing ampicillin and X-gal (40 μg/ml; Sigma).

Toxin screening. Selected *E. coli* RR1 transformants (Ap^r Tc^s) from shotgun cloning experiments were screened for toxin production by colony immunoblot analysis. The colonies were replica plated onto nitrocellulose membrane circles (0.45-μm pore size, 82-mm diameter; Schleicher & Schuell Inc., Keene, N.H.), soaked in 0.5 N NaOH, and washed with 0.1 M HCl. Membranes were incubated first in diluted enterotoxin C_1 antiserum and then exposed to alkaline phosphatase-conjugated anti-rabbit immunoglobulin G prepared in goats (1:500 dilution; Sigma). Antigen-antibody complexes were detected by the indoxyl phosphate-Nitroblue Tetrazolium indicator system (8). Toxin production by clones and subclones was confirmed by Ouchterlony immunodiffusion (20).

Toxin purification. Staphylococcus-derived enterotoxin C_1 was obtained from cultures of *S. aureus* MN Don grown in dialyzable beef heart medium (34). Cloned toxin was obtained by growing *E. coli* clones or subclones in LB broth containing ampicillin (40 μg/ml). Preliminary testing revealed that nearly 100% of toxin in *E. coli* cultures was cell associated (probably in the periplasm). The cultures (2,400 ml) were precipitated for 2 days (4°C) after mixing with 4 volumes of ethanol. Precipitates were dried and redissolved in distilled water. Enterotoxin C_1 was partially purified by preparative thin-layer isoelectric focusing (34) in a pH gradient of 3.5 to 10 by using commercial ampholytes (LKB Produkter, Bromma, Sweden). Areas of the gel bed containing proteins that reacted with enterotoxin C_1 antiserum were pooled and refocused in a narrow pH gradient (pH 7 to 9) to achieve additional purification.

Analytical gel electrophoresis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 15% acrylamide vertical gel slabs with the discontinuous buffer system of Laemmli (24). Analytical isoelectric focusing was performed in 5% polyacrylamide gel slabs with a pH gradient of 3.5 to 10 by using a commercial ampholyte preparation (LKB) as directed by the manufacturer (43).

Western blot analysis. After SDS-PAGE, proteins were transferred to 0.45-μm (pore size) nitrocellulose membranes (Schleicher & Schuell) in a Trans-Blot Cell (BioRad Laboratories, Richmond, Calif.). Electrophoretic transfer (anodal migration) was performed overnight (250 mA) in 25 mM Tris buffer, pH 8.3, containing 192 mM glycine and 20% (vol/vol) methanol. Residual protein-binding sites were blocked with gelatin. The nitrocellulose membranes were incubated in diluted enterotoxin C_1 antiserum that was previously absorbed with *E. coli* JM83(pUC13). The membranes were then incubated in alkaline phosphatase-conjugated anti-rabbit immunoglobulin G prepared in goats (Sigma). Antigen-antibody complexes were detected as described above (see Toxin screening).

Biological assays. Mitogenicity of staphylococcus-derived and cloned enterotoxin C_1 samples was assessed by using murine (BALB/c Wat) splenocytes as indicators of proliferation (30). Concanavalin A (Sigma) was used as a positive mitogenic control. [^{3}H]thymidine was purchased from Amersham Corp., Arlington Heights, Ill. Pyrogenicity and enhancement of host susceptibility to lethal endotoxin shock was determined by using American Dutch-belted rabbits (1.4 to 1.9 kg [body weight]) as described previously (21). Endotoxin was prepared from *Salmonella typhimurium* by the method of Westphal and Jann (42) and administered intravenously (i.v.) 4 h after the animals received enterotoxin C_1.

RESULTS

Cloning the enterotoxin C_1 gene (*entC*$_1$). DNA from *S. aureus* MN Don was used to construct a library in *E. coli*. Because evidence has been previously provided for both chromosomal and plasmid location of the gene (1; M. J. Betley and M. S. Bergdoll, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, D38, p. 49), total genomic DNA was used for cloning. Plasmids were not detected in *S. aureus* MN Don by routine screening procedures.

The genome was partially digested with *Mbo*I, and 5 to 10-kilobase (kb) DNA fragments were isolated and ligated to pBR328 previously treated with *Bam*HI and alkaline phosphatase. The DNA mixture was transformed into *E. coli* RR1, and approximately 1,000 transformants carrying recombinant plasmids were selected on the basis of antibiotic resistance. Two colonies producing enterotoxin C_1 were detected by reactivity with antiserum in immunoblots and Ouchterlony immunodiffusion. The recombinant plasmids carried by these clones were designated pMIN101 and pMIN102.

Subcloning and restriction analysis. The 12.0-kb plasmid pMIN101, containing a 7.1-kb insert, was chosen for

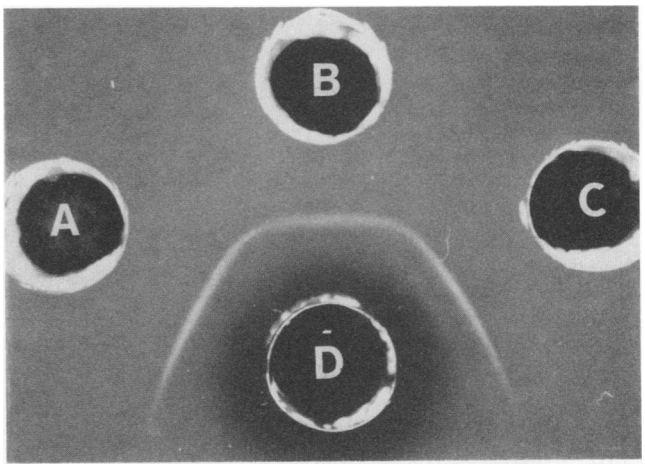

FIG. 2. Immunological reactivity of purified enterotoxin C_1 from (A) *E. coli* JM83(pMIN114), (B) *S. aureus* MN Don, and (C) *E. coli* RR1(pMIN101) with (D) enterotoxin C_1 rabbit antiserum. Wells of an Ouchterlony immunodiffusion slide were filled with 20 μl of toxin (20 μg) or antiserum, incubated for 4 h (37°C), and observed for precipitation lines of identity.

subcloning $entC_1$ (Fig. 1). Digestion of pMIN101 with *Hin*dIII produced fragments A (5.4 kb), B (4.6 kb), and C (2.0 kb). *Cla*I digested pMIN101 into fragments A (6.9 kb) and B (5.1 kb). Subcloning either *Hin*dIII B or *Cla*I A fragments into pUC13 (pMIN111 and pMIN112, respectively) and transforming the DNA into *E. coli* JM83 resulted in transformants expressing enterotoxin C_1. Digestion of *Hin*dIII-B with *Bgl*II or *Cla*I yielded DNA fragments of 3.3 and 1.5 kb, respectively, that contained $entC_1$. These fragments were cloned into pUC13, and the recombinants (pMIN113 and pMIN114) encoded toxin that was immunologically identical to that of *E. coli* RRI(pMIN101) and *S. aureus* MN Don (Fig. 2).

Biological activity of cloned enterotoxin C_1. Enterotoxins C_1 purified from cultures of *E. coli* JM83 (pMIN114) and *S. aureus* MN Don were compared for pyrogenicity and capacity to enhance susceptibility of rabbits to lethal endotoxin shock. Test rabbits were given intravenous injections of enterotoxin C_1 (5 μg/kg). Enterotoxins C_1 from *S. aureus* and *E. coli* were equally pyrogenic (Fig. 3). The average temperature rise in test rabbits was 1.25°C over the 4-h period. The pyrogenic response peaked at 4 h and was monophasic. Therefore, endotoxin contamination, which typically induces a biphasic temperature rise (13), did not contribute significantly to the pyrogenic response to cloned enterotoxin C_1. Both toxin preparations also enhanced susceptibility to lethal endotoxin shock; all three rabbits in each group receiving prior injections of enterotoxin C_1 succumbed within 48 h after receiving endotoxin (10 μg/kg).

Cloned enterotoxin C_1 was also compared with staphylococcus derived toxin for mitogenicity. Mouse splenocytes (5×10^5) were exposed to 1 μg of enterotoxin C_1 from *E. coli* JM83(pMIN114) or *S. aureus* MN Don for 4 days. At 18 h before harvesting, the cultures were pulsed with 1 μCi of [^{3}H]thymidine, and incorporation of radiolabel into cellular DNA was quantitated. Both toxin preparations stimulated similar mitogenic responses (Fig. 4).

Biochemical characterization of cloned enterotoxin C_1. Protein contents of toxin preparations purified from *S. aureus* MN Don and *E. coli* JM83(pMIN114) were compared by analytical isoelectric focusing. Staphylococcus-derived enterotoxin C_1 existed in two major forms (Fig. 5). Analysis of cloned toxin demonstrated proteins with pIs identical to those of proteins from *S. aureus*. Two additional, more acidic proteins present in cloned enterotoxin C_1 preparations were *E. coli* proteins since they were also present in similar preparations from *E. coli* JM83 clones not harboring pMIN114.

In SDS-PAGE, both forms of staphylococcus-derived enterotoxin C_1 migrated as a single band (Fig. 6). Preparations of cloned toxin also contained an identical-sized protein (26,000 molecular weight). This was likely the intact toxin since it reacted with the enterotoxin C_1 antiserum probe in Western blot analysis. *E. coli*-derived enterotoxin C_1 preparations also contained significant amounts of 26,500-

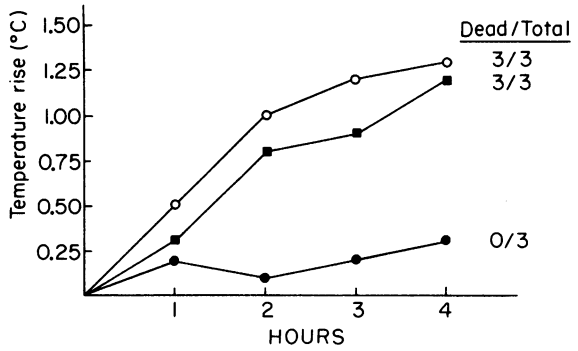

FIG. 3. Pyrogenicity and enhancement of rabbit susceptibility to lethal endotoxin shock. The mean (rectal) temperature rise of rabbits (three per group) was monitored after intravenous administration of enterotoxin C_1 (5 μg/kg) from *E. coli* JM83(pMIN114) (○), *S. aureus* MN Don (■), or an equivalent volume of diluent (●). After 4 h, endotoxin (10 μg/kg) was administered intravenously, and the animals were observed for 72 h for death.

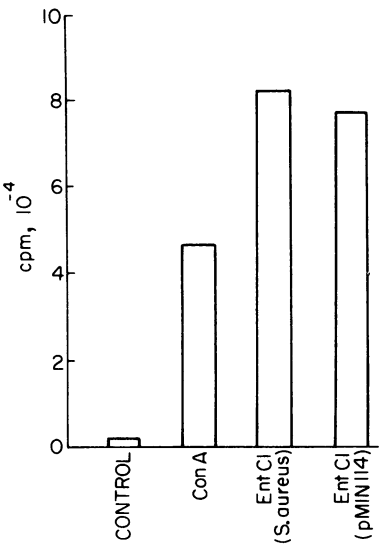

FIG. 4. Comparison of the mitogenic capacity of 1 μg of enterotoxins C_1 from *E. coli* JM83(pMIN114) and *S. aureus* MN Don and concanavalin A for murine splenocytes. Control wells received no stimulant. Incorporation of [^{3}H]thymidine into cellular DNA is expressed as the mean of four tests.

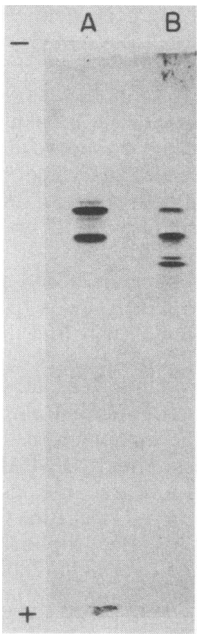

FIG. 5. Analytical isoelectric focusing of enterotoxin C_1 (20 μg) from (A) *S. aureus* MN Don and (B) *E. coli* JM83(pMIN114) in a pH gradient of 3 to 10.

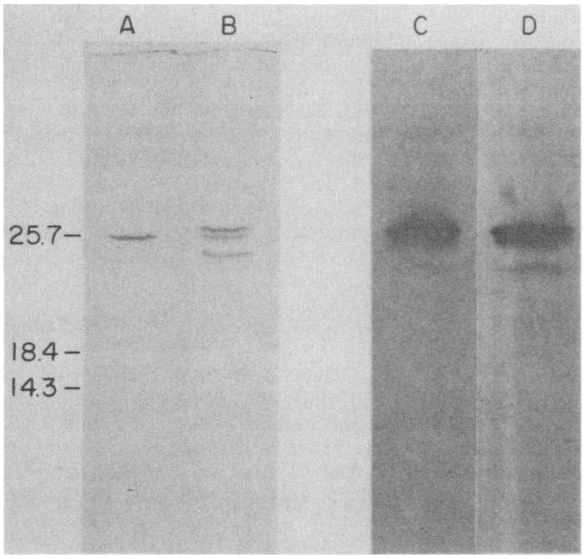

FIG. 6. SDS-PAGE and Western blot analysis of enterotoxin C_1 from (lanes A and C) *S. aureus* MN Don (0.5 μg) and (lanes B and D) *E. coli* JM83(pMIN114) (2.5 μg). Toxins were electrophoresed and stained with Coomassie blue (lanes A and B). The molecular masses of protein standards are given in kilodaltons. An identical set of proteins was transferred to nitrocellulose and probed with enterotoxin C_1 antiserum (lanes C and D).

and 24,800-molecular-weight proteins. The smaller protein reacted with the antiserum probe in Western blots and may represent a degradative product of toxin. The 26,500-molecular-weight nonreactive protein was probably an *E. coli* contaminant.

DISCUSSION

In this study, staphylococcal enterotoxin C_1 was cloned into *E. coli*. Subcloning enabled localization of the toxin gene *entC*$_1$ onto a 1.5-kb *Hin*dIII-*Cla*I DNA fragment that was expressed when inserted into the vector pUC13. Cloned toxin, produced by *E. coli* JM83 carrying this recombinant plasmid (pMIN114), was subjected to immunological, biochemical, and biological characterization.

Immunological identity between staphylococcus-derived enterotoxin C_1 and cloned toxin was confirmed by Ouchterlony immunodiffusion and Western blot analysis. The molecular weight of toxin purified from *S. aureus* MN Don and clones was determined to be 26,000, comparable to the value of 27,500 predicted by Schmidt and Spero (35) based on amino acid composition.

Enterotoxin C_1 is reported to be a basic protein with a pI of 8.6 (11). This value is consistent with our observations during preparative isoelectric focusing of enterotoxin from *S. aureus* MN Don and *E. coli* JM83(pMIN114). However, two charged species of enterotoxin C_1 could be distinguished by analytical isoelectric focusing in polyacrylamide gels. Charge heterogeneity is also observed with TSST-1 (9) and streptococcal pyrogenic exotoxin A (28).

Further evidence that pMIN114 contains the entire *entC*$_1$ structural gene was provided by biological activity studies of cloned toxin. These studies also confirmed that biological activities previously attributed to enterotoxin C_1 are due to the toxin and not a copurified staphylococcal contaminant. *E. coli*-derived enterotoxin C_1 was pyrogenic and enhanced susceptibility of rabbits to lethal endotoxin shock. The cloned toxin was also mitogenic for mouse splenocytes in vitro. The activity of cloned toxin in these assays was identical to that of purified enterotoxin C_1 from *S. aureus* MN Don. This finding, together with the lack of a biphasic fever response, suggested that endotoxin contamination was not responsible for the observed effects of cloned enterotoxin C_1 from *E. coli* JM83(pMIN114). Although emetic activity of cloned enterotoxin C_1 was not evaluated, all other biological activities were conserved. Thus, the cloned toxin also likely retains its emetic activity.

The data presented in this report are important for future studies on the structure-function relationship of this group of toxins. Such studies will depend in part on nucleotide sequence analysis of representative toxins of each family. Other investigators thus far have cloned staphylococcal enterotoxins A and B (6, 31), TSST-1 (22), and streptococcal pyrogenic exotoxin A (19).

LITERATURE CITED

1. **Altboum, Z., I. Hertman, and S. Sarid.** Penicillinase plasmid-linked genetic determinants for enterotoxins B and C_1 production in *Staphylococcus aureus*. Infect. Immun. **47**:514–521.
2. **Barsumian, E. L., C. M. Cunningham, P. M. Schlievert, and D. W. Watson.** 1978. Heterogeneity of group A streptococcal pyrogenic exotoxin type B. Infect. Immun. **20**:512–518.
3. **Bergdoll, M. S., R. N. Robbins, K. Weiss, C. R. Borja, I. Y. Huang, and F. S. Chu.** 1973. The staphylococcal enterotoxins: similarities. Contrib. Microbiol. Immunol. **1**:390–396.
4. **Bergdoll, M. S., and P. M. Schlievert.** 1984. Toxic shock syndrome toxin. Lancet **ii**:691.
5. **Bertani, G.** 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli. J. Bacteriol. **62**: 293–300.
6. **Betley, M. J., S. Lofdahl, B. N. Kreiswirth, M. S. Bergdoll, and R. P. Novick.** 1984. Staphylococcal enterotoxin A gene is associated with a variable genetic element. Proc. Natl. Acad. Sci. USA **81**:5179–5183.
7. **Betley, M. J., and J. M. Mekalanos.** 1985. Staphylococcal enterotoxin A is encoded by phage. Science **229**:185–187.
8. **Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich.** 1984. A rapid, sensitive method for detection of alkaline phosphatase conjugated anti-antibody on Western blots. Anal. Biochem. **136**:175–179.
9. **Blomster-Hautamaa, D. A., B. N. Kreiswirth, R. P. Novick, and P. M. Schlievert.** 1986. The resolution of highly purified toxic-shock syndrome toxin-1 (TSST-1) into two distinct proteins by isoelectric focusing. Biochemistry **25**:54–59.
10. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
11. **Borja, C. R., and M. S. Bergdoll.** 1967. Purification and partial characterization of enterotoxin C produced by *Staphylococcus aureus* strain 137. Biochemistry **6**:1467–1473.
12. **Brunson, K. W., and D. W. Watson.** 1974. Pyrogenic specificity of streptococcal exotoxins, staphylococcal enterotoxin, and gram-negative endotoxin. Infect. Immun. **10**:347–351.
13. **Clewell, D. B.** 1972. Nature of Col E_1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
14. **Dolman, C. E., and R. J. Wilson.** 1938. Experiments with staphylococcal enterotoxin. J. Immunol. **35**:13–30.
15. **Hewick, R. M., M. W. Hunkapiller, L. E. Hood, and W. J. Dreyer.** 1981. A gas-liquid solid phase peptide and protein sequenator. J. Biol. Chem. **256**:7990–7997.
16. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**: 193–197.
17. **Huang, I. Y., and M. S. Bergdoll.** 1970. The primary structure of staphylococcal enterotoxin B. III. The cyanogen bromide peptides of reduced and amino ethylated enterotoxin B, and the

complete amino acid sequence. J. Biol. Chem. **245**:3518–3525.
18. **Johnson, L. P., J. J. L'Italien, and P. M. Schlievert.** 1986. Streptococcal pyrogenic exotoxin type A (scarlet fever toxin) is related to *Staphylococcus aureus* enterotoxin B. Mol. Gen. Genet. **203**:354–356.
19. **Johnson, L. P., and P. M. Schlievert.** 1984. Group A streptococcal phage T12 carries the structural gene for pyrogenic exotoxin type A. Mol. Gen. Genet. **194**:52–56.
20. **Johnson, L. P., M. A. Tomai, and P. M. Schlievert.** 1986. Bacteriophage involvement in group A streptococcal pyrogenic exotoxin A production. J. Bacteriol. **166**:623–627.
21. **Kim, Y. B., and D. W. Watson.** 1970. A purified group A streptococcal pyrogenic exotoxin. Physicochemical and biological properties including the enhancement of susceptibility to endotoxin lethal shock. J. Exp. Med. **131**:611–628.
22. **Kreiswirth, B. N., S. Lofdahl, M. J. Betley, M. O'Reilly, P. M. Schlievert, M. S. Bergdoll, and R. P. Novick.** 1983. The toxic shock syndrome exotoxin strucutral gene is not detectably transmitted by a prophage. Nature (London) **305**:709–712.
23. **Kushner, S. R.** 1978. An improved method for transformation of *Escherichia coli* with ColE1 derived plasmids, p. 17. *In*: H. B. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier/North-Holland Publishing Co., Amsterdam.
24. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
25. **Macrina, F. L., J. L. Reider, S. S. Virgili, and D. J. Kopecko.** 1977. Survey of the extrachromosomal gene pool of *Streptococcus mutans*. Infect. Immun. **17**:215–226.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 167. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Messing, J.** 1979. A multi-purpose cloning system based on the single-stranded DNA bacteriophage M13. Recombinant DNA technical bulletin, NIH publication no. 79-99, p. 43–48. National Institutes of Health, Bethesda, Md.
28. **Nauciel, C., J. Blass, R. Mangalo, and M. Raynaud.** 1969. Evidence for two molecular forms of streptococcal erythrogenic toxin. Conversion to a single form by 2-mercaptoethanol. Eur. J. Biochem. **11**:160–164.
29. **Poindexter, N. J., and P. M. Schlievert.** 1985. The biochemical and immunological properties of toxic-shock syndrome toxin-1 (TSST-1) and association with TSS. J. Toxicol. Toxin Rev. **4**:1–39.
30. **Poindexter, N. J., and P. M. Schlievert.** 1985. Toxic-shock syndrome toxin-1-induced proliferation of lymphocytes: comparison of the mitogenic response of human, murine, and rabbit lymphocytes. J. Infect. Dis. **151**:65–72.
31. **Ranelli, D. M., C. L. Jones, M. B. Johns, G. J. Mussey, and S. A. Khan.** 1985. Molecular cloning of staphylococcal enterotoxin B gene in *Escherichia coli* and *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **82**:5850–5854.
32. **Schlievert, P. M., K. M. Bettin, and D. W. Watson.** 1977. Purification and characterization of group A streptococcal pyrogenic exotoxin type C. Infect. Immun. **16**:673–679.
33. **Schlievert, P. M., D. J. Schoettle, and D. W. Watson.** 1979. Purification and physicochemical and biological characterization of a staphylococcal pyrogenic exotoxin. Infect. Immun. **23**:609–617.
34. **Schlievert, P. M., K. N. Shands, B. B. Dan, G. P. Schmid, and R. D. Nishimura.** 1981. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic-shock syndrome. J. Infect. Dis. **143**:509–516.
35. **Schmidt, J. J., and L. Spero.** 1983. The complete amino acid sequence of staphylococcal enterotoxin C_1. J. Biol. Chem. **258**:6300–6306.
36. **Schwab, J. H., D. W. Watson, and W. J. Cromartie.** 1955. Further studies of group A streptococcal factors with lethal and cardiotoxic properties. J. Infect. Dis. **96**:14–18.
37. **Smith, B. G., and H. M. Johnson.** 1975. The effect of staphylococcal enterotoxins on the primary in vitro immune response. J. Immunol. **115**:575–578.
38. **Smith, H. O.** 1980. Recovery of DNA from gels. Methods Enzymol. **65**:371–380.
39. **Soberon, X., L. Covarrubias, and F. Bolivar.** 1980. Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. Gene **9**:287–305.
40. **Sugiyama, H., E. M. McKissic, M. S. Bergdoll, and B. Heller.** 1964. Enhancement of bacterial endotoxin lethality by staphylococcal enterotoxin. J. Infect. Dis. **114**:111–118.
41. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
42. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharide extraction with phenol-water and further application of the procedure. Methods Carbohydr. Chem. **5**:83–91.
43. **Winter, A., H. Perlmutter, and H. Davis.** 1975. Preparative flat-bed electrofocusing in a granulated gel with the LKB 2117 Multiphor. LKB Instruments, Stockholm.