JOURNAL OF BACTERIOLOGY, June 1989, p. 3068–3073
0021-9193/89/063068-06$02.00/0

Vol. 171, No. 6

Bacteriophage Association of Streptococcal Pyrogenic Exotoxin Type C

STEPHEN C. GOSHORN* AND PATRICK M. SCHLIEVERT

Department of Microbiology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

Received 20 October 1988/Accepted 4 March 1989

A gene encoding streptococcal pyrogenic exotoxin type C (SPE C) was isolated from bacteriophage DNA derived from *Streptococcus pyogenes* CS112. The gene, designated *speC2*, was shown to reside near the phage attachment site of phage CS112. A restriction endonuclease map of the CS112 phage was generated, and the location and orientation of the *speC2* gene were determined. Hybridization analyses of eight SPE C-producing strains revealed restriction fragment length polymorphism of the *speC* gene-containing DNA fragments and further showed that each *speC* was linked to a common CS112 phage-derived DNA fragment.

Streptococcal pyrogenic exotoxins (SPEs) are three antigenically distinct yet biochemically and biologically similar toxins. One or more of these toxins, designated types A, B, and C, are produced in more than 90% of tested *Streptococcus pyogenes* isolates (26). The SPEs are the causative agents of a toxic shock-like illness (8) and scarlet fever and may also be associated with strains causing delayed sequelae such as rheumatic fever and acute glomerulonephritis (26, 35).

The SPEs share many characteristics with the enterotoxins (Ents) and toxic shock syndrome toxin-1 (TSST-1) of *Staphylococcus aureus*. Thus far, the amino acid and nucleotide sequences have been determined for six of these toxins: SPE A (38) and SPE C (11), Ent A (4), Ent B (17), and Ent C1 (6), and TSST-1 (5). Computer-aided analysis of the mature toxin amino acid sequences indicates significant homology between each of the toxins except for TSST-1 (4, 11). Homology extends to the nucleotide sequences, and in particular, the structural genes for Ent B and Ent C1 are more than 70% homologous, and both appear to be closely related to SPE A (6). A lesser degree of sequence similarity is seen between SPE C and the other toxins (11); however, a few highly conserved regions are found in each of the toxins, with the exception of TSST-1.

It has been suggested by several researchers that the toxins share common ancestry and have been disseminated among and between strains of *S. pyogenes* and *S. aureus* by mobile elements such as bacteriophages and plasmids. The toxin structural genes for SPE A and Ent A have been demonstrated to reside on bacteriophages, although the majority of SPE A- and Ent A-producing strains appear to lack toxin-converting phages (3, 14). SPEs B and C have likewise been reported to be associated with bacteriophages (7, 15, 22). Plasmids encoding genes for Ents B and C1 have been reported (1, 27), although both toxins are more often chromosomally located.

Bacteriophage association of SPE C was first reported in 1979 by Colon-Whitt et al. (7). In that study, phage induced from *S. pyogenes* C203U was used to convert a nontoxigenic strain to SPE C production. Later, Johnson et al. (15) induced phage from *S. pyogenes* CS112 (formerly 9211S) to infect *S. pyogenes* K56 and form an SPE C-producing lysogen. Early attempts in our laboratory to isolate the SPE C structural gene from each of these phages were unsuccessful. We subsequently cloned the toxin gene (*speC*) from *S. pyogenes* T18P, which does not appear to harbor an inducible phage (22). In that study, we showed that *speC* hybridized to the attachment site-containing fragment of phage CS112 (10).

In this study, we were able to isolate an SPE C structural gene from temperate phage CS112. A physical map of the phage was generated, and the toxin gene was found to reside near to the phage attachment site. Hybridization of *S. pyogenes* DNA with a toxin gene-specific probe and a phage DNA-derived probe indicated that the toxin gene was linked to a common DNA fragment in each SPE C-producing strain.

MATERIALS AND METHODS

Bacterial strains and plasmids. The following *S. pyogenes* strains were used in hybridization analysis: CS112 (B^+ C^+), T18P (C^+), C203 (A^+ B^+ C^+), CS24 (SPE^-), P386 (B^+ C^+), 86628 (C^+), 86809 (B^+ C^+), 86749 (C^+), 87554 (C^+), S187 (A^+), and H1286 (A^+). Strains 86628, 86809, 86749, and 87554 were generously provided by Edward L. Kaplan, Department of Pediatrics, University of Minnesota. Streptococcal chromosomal DNA was prepared essentially by previously described methodology (29). *Escherichia coli* JM101 [Δ(*lac-pro*) *supE thi* F *traD36 proAB lacI*^{q}*Z* Δ*M15* (21)] was used as the recipient of DNA in transformation procedures. *E. coli* JM83 [*ara* Δ(*lac-pro*) *strA thi* φ80d*lacZ*Δ*M15* (20)] was used for propagation of vector plasmid pUC13 (36). Plasmid DNA was prepared by standard procedures and purified by CsCl-ethidium bromide density gradients.

Preparation of phage DNA. Cultures of *S. pyogenes* strains were grown overnight at 37°C in modified number 1 broths (37) in which serum was omitted. Number 1 broths were reinoculated (10% [vol/vol]) with the overnight cultures. After 1.5 h of incubation of the broths at 37°C, phage were induced by the addition of mitomycin C (Sigma Chemical Co., St. Louis, Mo.) to 0.1 μg/ml. Incubation was continued for 3 h, after which cells were removed by centrifugation (1,000 × *g*, 20 min). The supernatant fluids were stored overnight on ice. Phage particles were recovered from supernatant fluids by centrifugation (60,000 × *g*, 3 h). The pelleted particles were suspended in 20 mM Tris hydrochloride (pH 7.4)–20 mM EDTA–200 mM NaCl–200 μg of proteinase K (Sigma) per ml and incubated for 1 h at 65°C. DNA was purified by successive phenol, phenol-chloroform, and chloroform extractions.

* Corresponding author.

Antiserum preparation. Hyperimmune antiserum was prepared by immunization of American Dutch belted rabbits with purified SPE C (25).

Cloning procedures. Streptococcal DNA was purified from agarose gels before ligation to plasmid pUC13. Transformations were performed by the method of Kushner (18). Transformants were selected by ampicillin resistance.

Toxin production. *E. coli* clones containing recombinant plasmids were grown overnight in 5 ml of LB medium (2) containing 50 μg of ampicillin per ml. The cultures were precipitated for 24 h (−20°C) after the addition of 4 volumes of ethanol. Precipitates were dried, dissolved in 0.2 ml of H_2O, and tested for SPE C production by precipitation with SPE C antiserum in Ouchterlony immunodiffusion tests.

Restriction endonuclease mapping. Digests of phage CS112 DNA were cleaved with one or two enzymes, separated on agarose gels, and visualized by ethidium bromide staining. Digestion fragments were eluted from gels by a freeze extraction method (30) and redigested with a second enzyme. The sizes of the restriction fragments in kilobases were determined by the computer program of Duggleby et al. (9).

DNA hybridizations. DNA was digested to completion with restriction enzymes and separated on 0.7% agarose gels and transferred to Nytran membranes (Schleicher & Schuell, Inc., Keene, N.H.) as described previously (31). Prehybridization and hybridization were performed at 68°C in 6× SET (20× SET is 500 mM NaCl, 30 mM Tris hydrochloride, 2 mM EDTA)–0.1% (wt/vol) sodium PP_i–0.2% (wt/vol) sodium dodecyl sulfate–200 μg of heparin (Sigma) per ml (28). After 1 h of prehybridization, denatured probe DNA was added and allowed to hybridize for 24 h. Blots were washed twice for 20 min in 3× SET–0.2% (wt/vol) sodium dodecyl sulfate at 65°C before exposure to X-ray film.

Hybridization probes. Fragments of DNA to be used as probes were eluted from agarose gels and radiolabeled with [α-^{32}P]dATP by nick translation reaction (24). Unincorporated [α-^{32}P]dATP was separated from probe DNA by ethanol precipitation. Probe DNA was denatured by the addition of NaOH to 0.2 M and incubation at 68°C for 5 min.

RESULTS

Cloning of *speC2*. In an earlier study, we reported hybridization of an *speC*-specific probe to CS112 phage DNA (10). In that experiment, a 7.3-kilobase (kb) *Hin*dIII fragment containing the phage attachment site was specified. Initial attempts to clone this fragment were unsuccessful; recombinant plasmids containing the 7.3-kb *Hin*dIII fragment were not found.

In this study, the 7.3-kb *Hin*dIII fragment was eluted from an agarose gel, subdigested with *Hin*cII, and separated by electrophoresis. The largest *Hin*cII fragment (2.2 kb) was eluted from the gel and ligated with *Hin*dIII linkers. After digestion with *Hin*dIII, excess linker was removed by agarose gel electrophoresis. The 2.2-kb fragment, now containing *Hin*dIII sites at each terminus, was eluted from the gel, ligated to pUC13, and transformed into *E. coli* JM101. Cell lysates from transformants harboring the recombinant plasmid (pUMN5050) contained SPE C as detected by the reaction with SPE C antiserum in Ouchterlony immunodiffusion tests (Fig. 1). The precipitin line formed a line of identity with SPE C prepared from *S. pyogenes* T18P. The cloned toxin gene, *speC2*, did not appear to differ from the previously isolated, chromosomally located toxin gene, *speC*, as indicated by restriction analysis and Ouchterlony immunodiffusion tests.

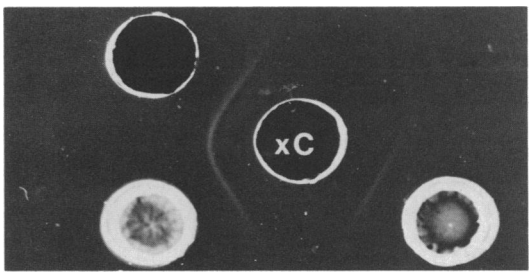

FIG. 1. Ouchterlony immunodiffusion reactivity of streptococcal and *E. coli*-derived SPE C. The well designated xC contained 20 μl of SPE C antiserum. The top left well contains purified SPE C from *S. pyogenes* T18P. The bottom wells contain cell lysates from *E. coli* JM101(pUMN5050).

Characterization of CS112 phage. DNA prepared from the CS112 phage was digested to completion with the restriction enzymes *Eco*RI, *Sal*I, and *Xba*I (Fig. 2). Each digest yielded four to six distinct restriction fragments along with submolar bands and diffuse bands of heterogeneous fragments which were found to be subsets of larger, heavier-staining fragments by DNA hybridization analysis (data not shown). In the *Xba*I digest, fragments B and C migrated identically, as indicated by the staining intensity and substantiated by subdigestion. When the fragment sizes were added for each digest, (discounting submolar and heterogeneous fragments), the sums were approximately equal—40.8 ± 0.1 kb. These restriction profiles are similar to the restriction profiles of *S. pyogenes* phages T12 (13) and SP24 (32). Both of these phages were determined to package DNA by the headful mechanism, first described for coliphage T4 (34) and also found in coliphage T1 (23) and *Salmonella typhimurium* phage P22 (12). In this mechanism, concatemers of phage DNA are produced on which packaging initiates at a precise site and proceeds by successive headfuls. A headful constitutes slightly more than one genome length, resulting in phage particles containing DNA that is terminally redundant and circularly permuted. Submolar and heterogeneous fragments are produced as a consequence of this type of packaging.

Restriction endonuclease cleavage map of CS112 phage. The cleavage sites of restriction enzymes *Eco*RI, *Sal*I, and *Xba*I were located on the CS112 phage genome through analysis of single and double digestion data and by redigestion of

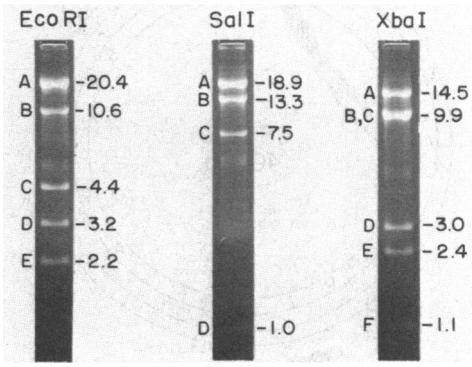

FIG. 2. Restriction endonuclease fragment profiles of CS112 phage after cleavage with *Eco*RI, *Sal*I, and *Xba*I. Fragments are designated with capital letters. Fragment sizes are indicated to the right of each digest in kilobase pairs.

TABLE 1. Redigestion of purified restriction fragments of CS112 phage DNA[a]

Purified fragment	Redigestion products generated with:		
	*Sal*I	*Eco*RI	*Xba*I
*Eco*RI			
A	D, 10.4, 9.0		D, F, 12.0, 4.3
B	C, 3.1		C, 0.4, 0.5
C	NC[b]		E, 1.9
D	NC		NC
E	NC		NC
*Sal*I			
A		D, E, 10.4, 3.1	B, D, F, 2.8, 2.0
B		C, 9.4	12.2, 1.0
C		NC	7.2, 0.5
D		NC	NC

[a] This table lists the restriction fragments obtained after digestion of purified restriction fragments with second enzymes. The capital letters refer to the assigned fragments in Fig. 2. Other fragments are designated by size (kilobase pairs).

[b] NC, Not cut.

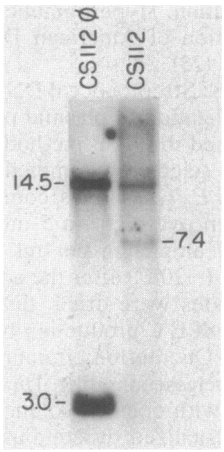

FIG. 4. Autoradiograph of *Xba*I-digested phage and chromosomal DNA derived from strain CS112 and hybridized with an *speC*-specific probe (Fig. 5, probe A). Approximate sizes are indicated in kilobase pairs.

purified restriction fragments with second enzymes (Table 1). For example, digestion of the *Sal*I A fragment with *Eco*RI resulted in four fragments: 10.4 kb, 3.1 kb, and the *Eco*RI D and *Eco*RI E fragments. The *Eco*RI D and E fragments mapped entirely within the *Sal*I A fragment. The 10.4-kb fragment also resulted from digestion of *Eco*RI-A with *Sal*I indicating that *Sal*I-A and *Eco*RI-A are overlapping fragments. The 3.1-kb and *Sal*I C fragments were *Sal*I subdigestion products of *Eco*RI-B. Thus, the *Sal*I A fragment overlaps with the *Eco*RI B fragment and is adjacent to the *Sal*I C fragment. Continuation of this analysis in a stepwise manner resulted in a circular phage map (Fig. 3). Ambiguities which arose in construction of the map were clarified by using restriction fragments as hybridization probes in Southern analysis of phage digests (data not shown).

Location of *speC2* gene and phage attachment sites on CS112 phage map. Chromosomal and phage DNAs from strain CS112 were digested to completion with the restriction endonuclease *Xba*I, separated on a 0.7% agarose gel, and transferred to a Nytran membrane. A 1.7-kb *speC*-specific DNA probe containing the entire *speC* structural gene and

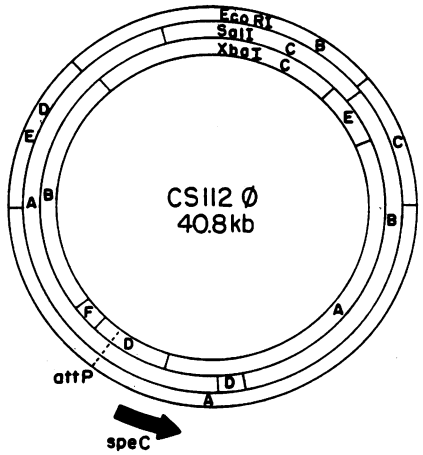

FIG. 3. Restriction endonuclease cleavage map of CS112 phage. Fragments are designated by their assigned letter (Fig. 2). The approximate locations of *speC* and *attP* are indicated.

including an internal *Xba*I site was allowed to hybridize to the membrane, and an autoradiogram was prepared (Fig. 4). In the phage digest, probe DNA hybridized to fragments of 14.5 kb (*Xba*I-A) and 3.0 kb (*Xba*I-D). In the chromosomal digest, probe DNA hybridized to a fragment of the same size as *Xba*I-A and a second fragment of 7.4 kb. The difference in hybridization pattern between the phage DNA and prophage-containing chromosomal DNA indicated that the phage attachment site (*attP*) resides within the *Xba*I D fragment. Upon integration of phage DNA, *attP*-containing fragments are split, with each portion joined to chromosomal DNA sequences. The 7.4-kb fragment that hybridized in the chromosomal digests was a junction fragment containing both bacterial and phage DNA. When a probe prepared from the 5′ portion of *speC* and terminating at the internal *Xba*I site was used, only the *Xba*I D and 7.4-kb chromosomal fragments hybridized (data not shown), thus indicating that the upstream and 5′ portions of *speC2* resided within *Xba*I-D. The location and orientation of *speC2* and the approximate location of *attP* are indicated on the phage map (Fig. 3).

Hybridization analysis of SPE C-producing strains. Although it has been established that strain CS112 harbors an *speC*-containing prophage, the majority of SPE C-producing strains do not appear to produce infectious *speC*-containing phage. In an effort to better understand the genetics of *speC*, we hybridized identical Nytran blots containing *Pst*I-digested chromosomal DNA to either a 0.6-kb fragment of DNA containing a portion of *speC* or a CS112 phage-derived DNA fragment which is linked to *speC2* on the CS112 phage (probe locations shown in Fig. 5). The *speC*-specific probe hybridized to one *Pst*I toxin gene-containing fragment in each strain (Fig. 6, lanes A), except for strains H1286, S187, and CS24 (lanes 9A, 10A, and 11A, respectively), which do not produce SPE C and did not hybridize. Of the eight strains containing *speC*, fragments of six distinct sizes were specified. Lanes B in Fig. 6 were hybridized with a probe derived from CS112 phage DNA, a 1.0-kb *Sal*I phage fragment which maps approximately 1.5 kb from *speC2* on the same side of the phage attachment site (probe B, Fig. 5). Each of the toxin gene-containing fragments which hybridized in lanes 1A through 8A also hybridized in lanes B to probe B. Strains H1286 and S187, though lacking *speC*, contained sequences which hybridized to probe B (lanes 9B

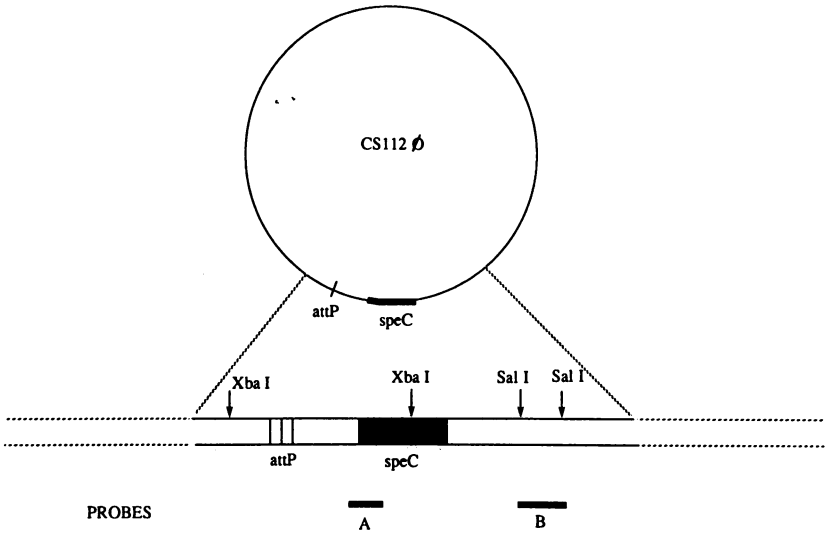

FIG. 5. Hybridization probe locations. Both probes were derived from CS112 phage DNA. Probe A contained approximately 450 bases of the toxin structural gene and 150 bases upstream. Probe B is a 1.0-kb *Sal*I fragment which mapped approximately 1.5 kb away from *speC*.

and 10B). Strain CS24 did not hybridize to either probe (lanes 11A and B).

DISCUSSION

In an earlier study, we reported hybridization of an *speC*-specific probe to CS112 phage DNA (10). A 7.3-kb phage *Hin*dIII fragment was specified, and it was noted that this fragment contained the phage attachment site (*attP*). Attempts to clone this fragment were unsuccessful. In this study, we were able to construct an SPE C-producing clone after subdigesting the *Hin*dIII fragment with *Hin*cII and isolating a 2.2-kb fragment containing the toxin gene. Our inability to clone the larger *Hin*dIII fragment may have been due to instability of the *attP* site or to the presence of a gene near *speC2* whose expression is lethal to *E. coli*. The clone, JM101(pUMN5050), produced SPE C which appeared antigenically identical by Ouchterlony immunodiffusion to SPE C from strain T18P. Restriction site polymorphism within the toxin structural genes was not detected between the T18P chromosome-derived *speC* and the CS112 phage-derived *speC2*.

Other phage-encoded bacterial toxins include *S. pyogenes* SPE A (14), *S. aureus* Ent A (3), and diphtheria toxin (19). In each of these, the toxin structural gene resides near the phage attachment site, and it has been suggested that the phage acquired the toxin gene by an aberrant excision event between a chromosomally located toxin gene and a closely inserted prophage. Considering that the phage CS112 attachment site maps less than 2.0 kb away from *speC2*, it is possible that CS112 phage acquired the toxin gene by an aberrant excision event.

We observed that toxin-converting phage are relatively rare or at least difficult to demonstrate in SPE C-producing strains. Similar observations have been reported for SPE A (16) and Ent A (3). In this study, we demonstrated the linkage of the SPE C structural gene with a CS112 phage-derived DNA fragment in all SPE C-producing strains tested. It is possible that CS112 phage acquisition of the toxin gene was a relatively recent event in which the bacterial *speC* and the flanking DNA, specified by probe B, were acquired simultaneously. However, the commonplace occurrence of *speC* restriction fragment length polymor-

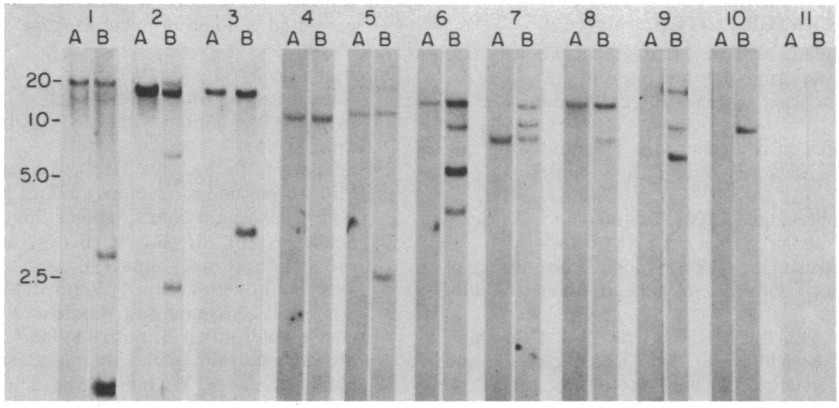

FIG. 6. Autoradiographs of *S. pyogenes Pst*I-digested chromosomal DNA from the following strains: 1, CS112; 2, T18P; 3, C203; 4, 86628; 5, 86729; 6, 87554; 7, 86809; 8, P386; 9, S187; 10, H1286; 11, CS24. Lanes designated A were hybridized with an *speC*-specific probe (Fig. 5, probe A). Lanes designated B were hybridized with a CS112 phage-specific probe (Fig. 5, probe B). Fragment sizes are indicated to the left of the autoradiographs in kilobase pairs.

phism argues that most, if not all, *speC* genes are or were at one time associated with a mobile genetic element. Hybridization of the SPE C-producing strains C203, T18P, and NY5 with whole labeled CS112 DNA yielded several hybridized fragments in each strain, although few fragments were found which comigrated with the hybridized fragments of strain CS112 (unpublished data). We hypothesize that the CS112 phage is responsible for the dissemination of *speC* among strains of *S. pyogenes* and now exists in a defective or altered state in the majority of SPE C-producing strains.

Defective phages are most likely formed by recombinational events leading to deletions of essential genes in the endogenous prophage. Noninducible phages may also result from aberrant integration into the chromosome following infection. The resulting prophage would be intact but would lack the proper terminal sequences required for excision. Recombination between CS112 phage and other endogenous phages could result in the formation of CS112-related phages having altered host ranges. One such event in which an infecting and an endogenous phage in *S. pyogenes* formed a viable recombinant phage has been reported (33).

If the assumption is made that phage CS112 acquired *speC* by an aberrant excision event within *S. pyogenes*, it should be possible to detect CS112 phage or relatives of CS112 phage both associated and unassociated with toxin, and it should also be possible to detect *speC* genes, or progenitors of *speC* genes, which are not adjacent to phage sequences. We found with high-stringency hybridization several fragments of DNA which are similar to the CS112 phage-derived probe (Fig. 6) but lack the *speC* gene. These fragments may represent portions of CS112-related phages which are not associated with *speC*. Thus far, we have analyzed 20 strains and have not been able to find an *speC* gene that is not adjacent to CS112 phage sequences. Similar findings have been reported for SPE A and Ent A (3, 16). The toxin gene acquisition events may have occurred outside of the present species boundary or occurred sufficiently long ago that progenitor toxin genes have evolved away from being detectable by high-stringency hybridization.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant HL36611 from the National Heart, Lung, and Blood Institute. S.G. was supported by Public Health Service training grant 5T32 CA09138 from the National Cancer Institute.

We thank Timothy Leonard for artwork and photography.

LITERATURE CITED

1. **Altboum, Z., I. Hertman, and S. Sarid.** 1985. Penicillinase plasmid-linked genetic determinants for enterotoxins B and C1 production in *Staphylococcus aureus*. Infect. Immun. **47**:514–521.
2. **Bertani, G.** 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. **62**:293–300.
3. **Betley, M. J., and J. J. Mekalanos.** 1985. Staphylococcal enterotoxin A is encoded by phage. Science **229**:185–187.
4. **Betley, M. J., and J. J. Mekalanos.** 1988. Nucleotide sequence of the type A staphylococcal enterotoxin gene. J. Bacteriol. **170**: 34–41.
5. **Blomster-Hautamaa, D. A., B. N. Kreiswirth, J. S. Kornblum, R. P. Novick, and P. M. Schlievert.** 1986. The nucleotide and partial amino acid sequence of toxic shock syndrome toxin-1. J. Biol. Chem. **261**:15783–15786.
6. **Bohach, G. A., and P. M. Schlievert.** 1987. Nucleotide sequence of the staphylococcal enterotoxin C1 gene and relatedness to other pyrogenic toxins. Mol. Gen. Genet. **209**:15–20.
7. **Colon-Whitt, A., R. S. Whitt, and R. M. Cole.** 1979. Production of an erythrogenic toxin (streptococcal pyrogenic exotoxin) by a nonlysogenized group A streptococcus, p. 64–65. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks Ltd., Chertsey, Surrey, England.
8. **Cone, L. A., D. R. Woodard, P. M. Schlievert, and G. S. Tomory.** 1987. Clinical and bacteriologic observations of a toxic shock-like syndrome due to *Streptococcus pyogenes*. N. Engl. J. Med. **317**:146–149.
9. **Duggleby, R. G., H. Kinns, and J. I. Rood.** 1981. A computer program for determining the size of DNA restriction fragments. Anal. Biochem. **110**:49–55.
10. **Goshorn, S. C., G. A. Bohach, and P. M. Schlievert.** 1988. Cloning and characterization of the gene, *speC*, for pyrogenic exotoxin type C from *Streptococcus pyogenes*. Mol. Gen. Genet. **212**:66–70.
11. **Goshorn, S. C., and P. M. Schlievert.** 1988. Nucleotide sequence of streptococcal pyrogenic exotoxin type C. Infect. Immun. **56**:2518–2520.
12. **Jackson, E. N., D. A. Jackson, and R. J. Deans.** 1978. EcoRI analysis of bacteriophage P22 DNA. J. Mol. Biol. **118**:365–388.
13. **Johnson, L. P., and P. M. Schlievert.** 1983. A physical map of the group A streptococcal pyrogenic exotoxin bacteriophage T12 genome. Mol. Gen. Genet. **189**:251–255.
14. **Johnson, L. P., and P. M. Schlievert.** 1984. Group A streptococcal phage T12 carries the structural gene for pyrogenic exotoxin A. Mol. Gen. Genet. **194**:52–56.
15. **Johnson, L. P., P. M. Schlievert, and D. W. Watson.** 1980. Transfer of group A streptococcal pyrogenic exotoxin production to nontoxigenic strains by lysogenic conversion. Infect. Immun. **28**:254–257.
16. **Johnson, L. P., M. A. Tomai, and P. M. Schlievert.** 1986. Bacteriophage involvement in group A pyrogenic exotoxin A production. J. Bacteriol. **166**:623–627.
17. **Jones, C. L., and S. A. Khan.** 1986. Nucleotide sequence of the enterotoxin B gene from *Staphylococcus aureus*. J. Bacteriol. **166**:29–33.
18. **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.
19. **Laird, W., and N. Groman.** 1976. Orientation of the *tox* gene in the prophage of corynebacteriophage beta. J. Virol. **19**:228–231.
20. **Messing, J.** 1979. A multi-purpose cloning system based on the single-stranded DNA bacteriophage M13, p. 43–48. Recombinant DNA technical bulletin. NIH publication no. 79-99. National Institutes of Health, Bethesda, Md.
21. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
22. **Nida, S. K., and J. J. Ferretti.** 1982. Phage influence on the synthesis of extracellular toxins in group A streptococci. Infect. Immun. **36**:745–750.
23. **Ramsay, N., and D. A. Ritchie.** 1980. A physical map of the permuted genome of bacteriophage T1. Mol. Gen. Genet. **179**: 669–675.
24. **Rigby, P. W. J., M. Dieckman, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
25. **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.
26. **Schlievert, P. M., K. M. Bettin, and D. W. Watson.** 1979. Production of pyrogenic exotoxin by groups of streptococci: association with group A. J. Infect. Dis. **140**:676–681.
27. **Shafer, W. M., and J. J. Iandolo.** 1979. Genetics of staphylococcal enterotoxin B in methicillin-resistant isolates of *Staphylococcus aureus*. Infect. Immun. **25**:902–911.
28. **Singh, L., and K. W. Jones.** 1984. The use of heparin as a simple cost-effective means of controlling background in nucleic acid hybridization procedures. Nucleic Acids Res. **12**:5627–5638.
29. **Skjold, S. A., P. G. Quie, L. A. Fries, M. Burnham, and P. P. Cleary.** 1987. DNA fingerprinting of *Streptococcus zooepidemicus* (Lancefield group C) as an aid to epidemiological

study. J. Infect. Dis. **155**:1145–1150.
30. **Smith, H. O.** 1980. Recovery of DNA from gels. Methods Enzymol. **65**:371–380.
31. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
32. **Spanier, J. G., and P. P. Cleary.** 1983. A restriction map and analysis of the terminal redundancy in the group A streptococcal bacteriophage SP24. Virology **130**:502–513.
33. **Spanier, J. G., and P. P. Cleary.** 1983. A DNA substitution in the group A streptococcal bacteriophage SP24. Virology **130**: 514–522.
34. **Streisinger, G., R. S. Edgar, and G. H. Denhardt.** 1964. Chromosome structure in phage T4. I. Circularity of the linkage map. Proc. Natl. Acad. Sci. USA **51**:775–779.
35. **Veasy, L. G., S. E. Wiedmeier, G. S. Orsmond, H. D. Ruttenberg, M. M. Boucek, S. J. Roth, V. F. Tait, J. A. Thompson, J. A. Daly, E. L. Kaplan, and H. R. Hill.** 1987. Resurgence of acute rheumatic fever in the intermountain area of the United States. N. Engl. J. Med. **316**:421–427.
36. **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.
37. **Wannamaker, L. W., S. Almquist, and S. Skjold.** 1973. Intergroup phage reactions and transduction between group C and group A streptococci. J. Exp. Med. **137**:1338–1353.
38. **Weeks, C. R., and J. J. Ferretti.** 1986. Nucleotide sequence of the type A streptococcal exotoxin (erythrogenic toxin) gene from *Streptococcus pyogenes* bacteriophage T12. Infect. Immun. **52**:144–150.