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Staphylococcal Enterotoxin B Gene Is Associated with a Discrete Genetic Element

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The chromosomal location of the enterotoxin B gene in *Staphylococcus aureus* is unknown. Southern hybridization analysis of the chromosomal DNA from several enterotoxin B (SEB)-producing strains has shown that at least 26.8 kilobases (kb) of DNA is associated with the enterotoxin B gene (*entB*). We have found that one end of the *entB* element is located approximately 1.5 kb downstream of the *entB* gene. The chromosomal region adjacent to this end of the *entB* element was found to be homologous in several SEB-producing (SEB^+) and SEB-nonproducing (SEB^-) *S. aureus* strains. The chromosomes of all the SEB^+ strains studied were homologous for at least 24 kb upstream of the *entB* gene. Some naturally occurring SEB^- strains lacked the entire *entB* element, while others showed variable homology to the region upstream of the *entB* gene. These data suggest that the *entB* gene is part of a discrete genetic element that is at least 26.8 kb in size.

Staphylococcal enterotoxins are a group of extracellular proteins that are responsible for staphylococcal food poisoning. These enterotoxins are similar in their structure and mechanism of action and are classified into six serological groups, designated A, B, C_1, C_2, D, and E (2). The exact mechanism of action of these toxins is not fully understood, but they probably cause vomiting and diarrhea by an emetic action on the abdominal viscera and by inhibition of water absorption in the intestine (2, 4, 31).

Staphylococcal enterotoxin B (SEB) consists of a single polypeptide chain and has a molecular weight of 28,336 (14, 17). A precursor to SEB having a molecular weight of 32,000 has been identified (17, 25, 32). The mature toxin consists of 239 amino acid residues and shares nucleotide and amino acid sequence homology with staphylococcal enterotoxin C_1 and streptococcal pyrogenic exotoxin A (15, 34). The enterotoxin B gene (*entB*) has been cloned from *Staphylococcus aureus* S6, and its nucleotide sequence was reported (17, 25).

Early studies on the genetics of SEB production demonstrated that the *entB* gene cotransduced with the markers for methicillin resistance and tetracycline resistance at a high frequency (9). In addition, cotransduction of the *entB* gene with a small plasmid, pSN2, was also observed (27–29). However, the *entB* gene is not linked to any of these resistance markers (19, 30) and has been shown to be a chromosomally linked trait (25, 27, 28). In addition, transformation and hybridization analyses have demonstrated that pSN2 does not play any role in SEB production (18). Earlier experiments involving transformation, transduction, and mutation analysis suggest that the *entB* gene is structurally unstable and possibly part of a mobile genetic element such as a phage or a transposon (4, 9, 18, 25, 28, 29). In this paper we present DNA hybridization data suggesting that the *entB* gene is part of a discrete genetic element.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1.

Preparation of chromosomal and plasmid DNA. Chromosomal DNA was purified from *S. aureus* by a modification of a previously described method (7). Plasmid DNA was isolated from *Escherichia coli* by CsCl-ethidium bromide density gradient centrifugation of cleared lysates (8). Restriction endonuclease fragments from plasmid DNA were purified by electrophoresis through 5% polyacrylamide gels, followed by crushing and elution (23).

Cloning of *S. aureus* DNA. *S. aureus* chromosomal DNA was digested with *Bgl*II, and the fragments were centrifuged through a 10 to 30% sucrose gradient (22) in a Beckman SW-41 rotor for 19 h at 39,000 rpm. Fractions of 400 μl were collected, and 30-μl portions of each fraction were subjected to electrophoresis through a 0.7% agarose gel with Tris-borate-EDTA buffer (90 mM Tris, 90 mM borate, 2 mM EDTA, pH 8.3). The fractions containing fragments in the 10-kilobase (kb) size range were ethanol precipitated and ligated to *Bam*HI-digested pUC19 DNA, which had been treated with bacterial alkaline phosphatase (purchased from Bethesda Research Laboratories, Gaithersburg, Md.). The ligation products were used to transform *E. coli* JM83, which had been made competent by the method of Hanahan (13), and the transformed cells were plated on LB agar containing ampicillin (50 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-GAL) (20 μg/ml) (13). *E. coli* clones were selected by colony hybridization as described before (22).

Southern hybridization analysis. Chromosomal DNA (4 to 5 μg) from *S. aureus* strains was digested with restriction endonucleases (Bethesda Research Laboratories) and electrophoresed through 0.7% agarose gels in Tris-acetate-EDTA buffer (40 mM Tris-acetate, 2 mM EDTA). The gels were subjected to Southern hybridization analysis at 68°C with restriction fragments labeled either at their 5′ ends with [γ-^{32}P]ATP or with [α-^{32}P]dATP by the random primer method as probes (12, 22).

RESULTS

Examination of SEB^+ and SEB^- strains with probes flanking the *entB* gene. Southern hybridization analysis was performed on several SEB^+ and SEB^- *S. aureus* strains with DNA fragments derived from the plasmid pSK155 as probes. The plasmid pSK155 is a pBR322 derivative containing the cloned *entB* gene on a 6-kb *Hin*dIII fragment (25).

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
S. aureus		
S6	SEB^+	M. S. Bergdoll (18)
DU4916	SEB^+	R. P. Novick (9)
COL	SEB^+	J. J. Iandolo
10-275	SEB^+	J. J. Iandolo
FRI277	SEB^+	M. S. Bergdoll
FRI279	SEB^+	M. S. Bergdoll
57dk	SEB^+	J. J. Iandolo
D256	SEB^+	J. J. Iandolo
RN450	NCTC 8325 cured of prophages, SEB^-	R. P. Novick (24)
RN1030	RN450 *recA* (ϕ11), SEB^-	R. P. Novick (24)
RN1541	112 NG-1, SEB^-	R. P. Novick (35)
RN616	1019, SEB^-	R. P. Novick
S6-R	Spontaneous deletion mutant of S6, SEB^-	M. S. Bergdoll (10)
DU4916 (treated)	DU4916 cured of SEB production, SEB^-	R. P. Novick (9)
U320	Heterologous *S. aureus* strain, SEB^-	S. Lofdahl
E. coli		
JM83		Bethesda Research Laboratories
SK405	JM83(pSK405)	This work
Plasmids		
pUC19	Ap^r, vector	Bethesda Research Laboratories
pSK155	Ap^r, pBR322 carrying the *entB* gene	25
pSK405	Ap^r	This work

The location of the probes on the restriction map of pSK155 is shown in Fig. 1. Chromosomal DNA from SEB^+ and SEB^- strains was digested with *Hin*dIII and hybridized to these probes. All the SEB^+ strains contained a common 6-kb *Hin*dIII fragment to which all the probes hybridized (Fig. 2 and 3, and data not shown). In addition, two SEB^+ strains, DU4916 and 57dk, had additional *Hin*dIII fragments of 3.0 and 3.2 kb, respectively, to which all the probes hybridized except the *entB* gene-specific probes. The probes showed variable homology to these two *Hin*dIII fragments, with the greatest homology observed with the 950-base-pair (bp) *Hin*cII and 700-bp *Eco*RI probes (pSK155 map positions −0.85 to 0.1 and −1.8 to −1.1, respectively) located just upstream of the *entB* gene. In contrast to most of the SEB^+ strains, the SEB^- strains had variable homology with the probes derived from pSK155 (Fig. 2 and 3, and data not shown). These strains were shown to lack the *entB* gene by Southern hybridization analysis with the 150-bp *Taq*I probe specific for the *entB* structural gene (25). Two of the SEB^- strains, RN1541 and RN616, contained *Hin*dIII fragments of 2.7 and 3.0 kb, respectively, with strong homology to probes derived from regions flanking the *entB* gene (Fig. 2 and 3, and data not shown). The 10-kb *Hin*dIII fragment observed with RN616 was due to partial digestion of this DNA by

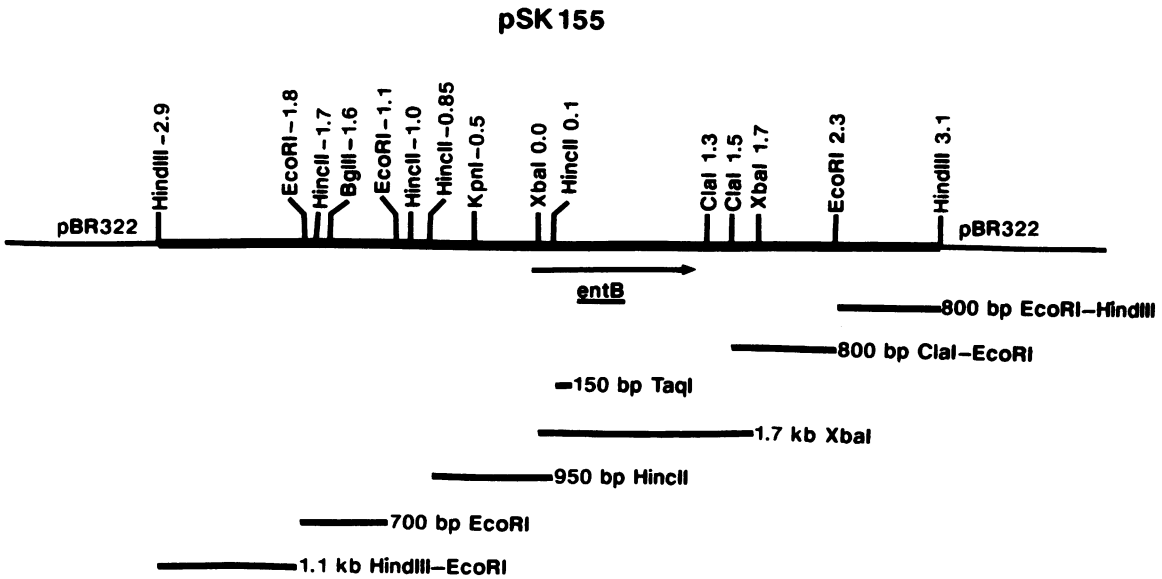

FIG. 1. Restriction map of pSK155. Map position 0.0 corresponds to the start of the *entB* gene. Bars below the map indicate the location of DNA fragments used as probes in this study. Map coordinates are given in kilobases.

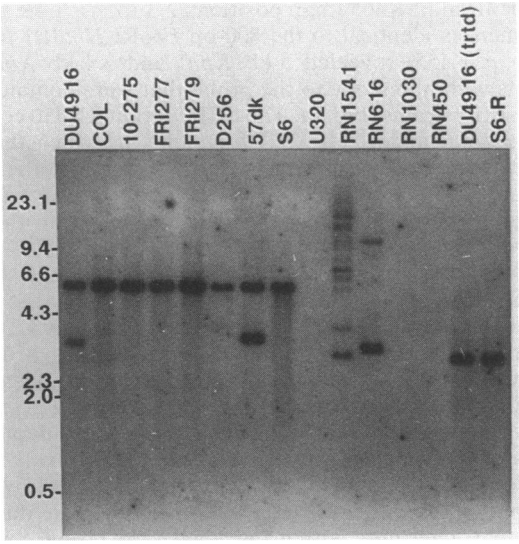

FIG. 2. Southern hybridization analysis of *S. aureus* strains with the 700-bp *Eco*RI fragment from pSK155 (map positions −1.8 to −1.1) as the probe. Chromosomal DNA from designated strains was digested with *Hin*dIII and analyzed. Bars represent lambda DNA *Hin*dIII size markers in kilobase pairs. The 10-kb band seen with RN616 and the multiple bands seen with RN1451 are due to partial digestion of these DNAs.

*Hin*dIII. Strain RN1541 had an additional *Hin*dIII fragment of 2.3 kb that hybridized strongly to the 800-bp *Cla*I-*Eco*RI and 1.7-kb *Xba*I probes (pSK155 map positions 1.5 to 2.3 and 0.0 to 1.7, respectively). The 800-bp *Cla*I-*Eco*RI probe also hybridized strongly to the SEB$^-$ strains RN1030 and DU4916 (treated) (data not shown). Two deletion mutants of parental SEB$^+$ strains, DU4916 (treated) and S6-R, had strong homology to the 700-bp *Eco*RI probe (Fig. 2). In all the other cases when a probe hybridized to an SEB$^-$ strain,

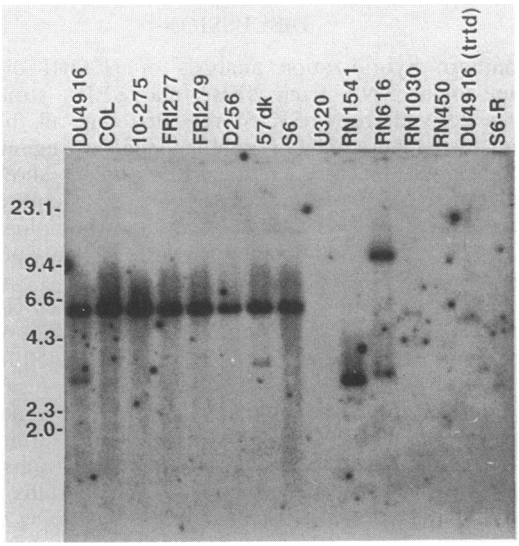

FIG. 3. Southern hybridization analysis of *S. aureus* strains with the 1.1-kb *Hin*dIII-*Eco*RI fragment from pSK155 (map positions −2.9 to −1.8) as the probe. Chromosomal DNA from designated strains was digested with *Hin*dIII and analyzed. Bars represent lambda DNA *Hin*dIII size markers in kilobase pairs. The 10-kb band seen with strain RN616 is due to partial digestion of this DNA.

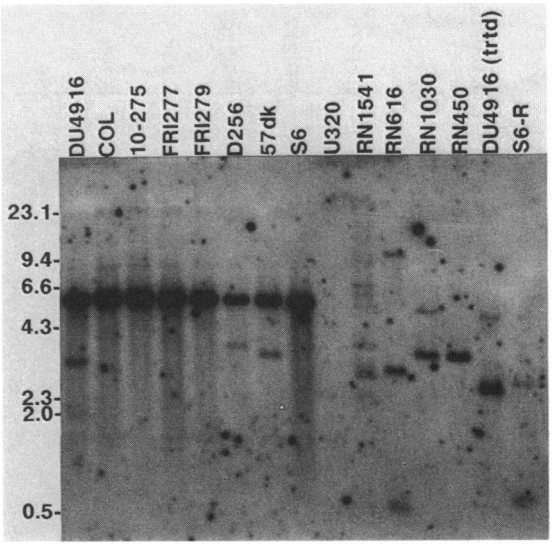

FIG. 4. Southern hybridization analysis of SEB$^+$ and SEB$^-$ *S. aureus* strains with the 800-bp *Eco*RI-*Hin*dIII fragment from pSK155 (map positions 2.3 to 3.1) as the probe. Chromosomal DNA from the designated strains was digested with *Hin*dIII and hybridized to the probe as described in the text. Bars indicate bacteriophage lambda DNA *Hin*dIII markers in kilobase pairs. The multiple bands seen with strain RN1541 are due to partial digestion of the DNA. The faint, slower-moving bands observed with many SEB$^-$ strains are probably also due to partial digestion of these DNAs.

the signal was weak, indicating partial homology to the chromosome of that strain. Only one probe, the 800-bp *Eco*RI-*Hin*dIII fragment from pSK155 (map positions 2.3 to 3.1), hybridized to all the SEB$^-$ strains except U320 (Fig. 4). Interestingly, this was the only probe which hybridized to strain RN450, which is a well-characterized derivative of NCTC 8325 that has been cured of all known prophages (24).

Analysis of the chromosomal sequences present upstream and downstream of the *entB* gene. The chromosomal region upstream of the *entB* gene was examined by using the 1.1-kb *Hin*dIII-*Eco*RI probe from pSK155 (map positions −2.9 to −1.8) as the probe. All the SEB$^+$ strains had common 24-kb *Kpn*I and 16-kb *Bgl*II fragments with homology to the probe (Fig. 5). This probe did not hybridize to the SEB$^-$ strain RN450. Analysis of the region downstream of the *entB* gene with the 800-bp *Eco*RI-*Hin*dIII fragment from pSK155 (map position 2.3 to 3.1) as the probe revealed 9.7-kb *Bgl*II and 3.5-kb *Cla*I fragments with homology to the probe that were common to all the SEB$^+$ strains (Fig. 6). In addition, the SEB$^-$ strain RN450 contained 8-kb *Bgl*II and 4.8-kb *Cla*I fragments with homology to this probe.

Cloning of the 9.7-kb *Bgl*II fragment from *S. aureus* S6. In order to compare the DNA sequences present further downstream of the *entB* gene in various *S. aureus* strains, a 9.7-kb *Bgl*II fragment from strain S6 to which the 800-bp *Eco*RI-*Hin*dIII fragment from pSK155 hybridized (Fig. 6) was cloned into *E. coli* by using pUC19 as the vector plasmid. Clones were selected by colony hybridization with the 3.4-kb *Eco*RI fragment from pSK155 (map positions −1.1 to 2.3) as the probe. Those colonies that gave strong signals were confirmed to contain the desired DNA fragment by Southern hybridization analysis of *Eco*RI-*Hin*dIII double digests of purified plasmid DNA with the 800-bp *Eco*RI-*Hin*dIII fragment as the probe. Two plasmids, pSK405 and pSK406, had an 800-bp *Eco*RI-*Hin*dIII fragment in common

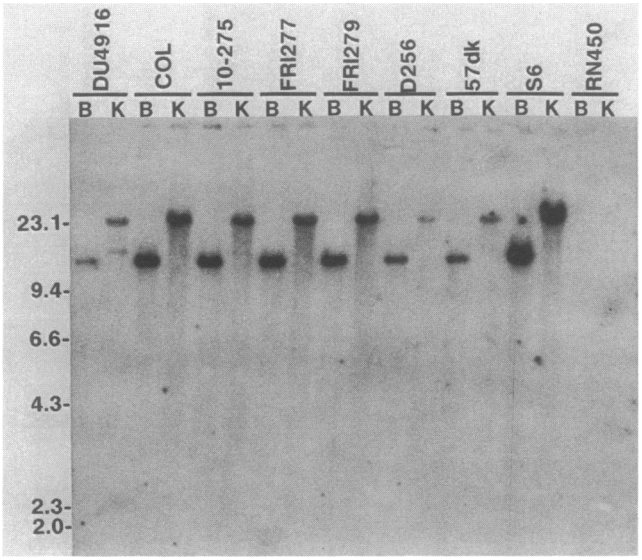

FIG. 5. Southern hybridization analysis of SEB$^+$ *S. aureus* strains with the 1.1-kb *Hin*dIII-*Eco*RI fragment from pSK155 (map positions −2.9 to −1.8) as the probe. Chromosomal DNA from the indicated SEB$^+$ strains was digested with *Bgl*II (B) or *Kpn*I (K) and analyzed as described in the text. Bars indicate lambda DNA *Hin*dIII size markers in kilobase pairs.

with pSK155 to which the probe hybridized, confirming that the desired DNA fragment had been cloned (data not shown).

Analysis of the chromosomal regions downstream of the *entB* gene with probes from pSK405. Probes were derived from pSK405 for use in analyzing the chromosomal region further downstream of the *entB* gene. The restriction map of pSK405 and the location of the probes on the map are shown in Fig. 7. This plasmid contains DNA that is common with that in pSK155 from map positions −1.6 to 3.1. It also contains an additional 5 kb of DNA. Analysis of *Kpn*I and *Xba*I digests of SEB$^+$ strains by using the 800-bp *Eco*RI

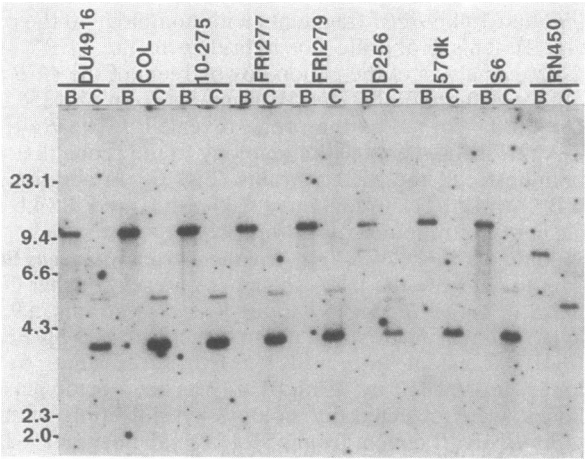

FIG. 6. Southern hybridization analysis of SEB$^+$ *S. aureus* strains with the 800-bp *Eco*RI-*Hin*dIII fragment from pSK155 (map positions 2.3 to 3.1) as the probe. Chromosomal DNA from the indicated SEB$^+$ strains was digested with *Bgl*II (B) or *Cla*I (C) and analyzed. Bars indicate lambda DNA *Hin*dIII size markers in kilobase pairs.

probe from pSK405 (map positions 2.3 to 3.1; note that this fragment is identical to the 800-bp *Eco*RI-*Hin*dIII fragment from pSK155) revealed 5-kb *Kpn*I and 3.1-kb *Xba*I fragments with homology to the probe that were common to all the SEB$^+$ strains (Fig. 8). The SEB$^-$ strain RN450 contained 25-kb *Kpn*I and 12-kb *Xba*I fragments to which the probe hybridized. Analysis of the *Eco*RI digests of other SEB$^-$ strains with the same probe revealed *Eco*RI fragments ranging from 800 bp to 4.4 kb with homology to the probe (data not shown).

Southern hybridization analysis of *Hin*dIII and *Xba*I digests of SEB$^+$ and SEB$^-$ strains by using the 950-bp *Kpn*I-*Cla*I fragment from pSK405 (map positions 4.1 to 5.05) revealed that all the strains except U320 had a common 2.4-kb *Hin*dIII fragment to which the probe hybridized (Fig. 9A and B). All the SEB$^+$ strains had a 3.1-kb *Xba*I fragment to which this probe hybridized, whereas considerable size heterogeneity of *Xba*I fragments was seen with the SEB$^-$ strains (Fig. 9A and B). The additional faint bands observed in lanes containing *Xba*I digests in Fig. 9B were due to the fact that *Xba*I cuts within the *Kpn*I-*Cla*I probe, and these bands represent an adjacent *Xba*I fragment with homology to the probe.

Subsequent analysis of *Hin*dIII and *Xba*I digests of SEB$^+$ and SEB$^-$ strains with the 2.1-kb *Hin*dIII fragment from pSK405 (map positions 6.0 to 8.1; note that the *Hin*dIII site at position 8.1 is present in the pUC19 vector and not in the cloned *S. aureus* DNA) as the probe revealed 3.4-kb *Hin*dIII and 3.1-kb *Xba*I fragments with homology to the probe that were common to all the strains except U320 (Fig. 10A and B). Surprisingly, additional *Hin*dIII fragments of 700 bp, 1.6 kb, and 2.2 kb and an additional *Xba*I fragment of 4.4 kb with homology to the probe were present in all the SEB$^+$ strains except S6 (Fig. 10A). However, the extra *Hin*dIII fragments were not observed with any SEB$^-$ strain except U320 (Fig. 10A and B). The additional *Xba*I fragment was also present in the SEB$^-$ strain U320. Two additional *Xba*I fragments present in the SEB$^-$ strain RN1541 were due to partial digestion of this DNA with *Xba*I.

DISCUSSION

Southern hybridization analysis of *Hin*dIII digests of chromosomal DNA from SEB$^+$ and SEB$^-$ strains with probes derived from pSK155 revealed that all the SEB$^+$ strains tested were highly homologous in this region of their chromosomes. In contrast, the SEB$^-$ strains showed size heterogeneity of the *Hin*dIII fragments to which the probes hybridized, as well as various degrees of homology to the probes (Fig. 2 to 4, and data not shown). The only probe that hybridized to the SEB$^-$ strain RN450, which has been cured of all known prophages, was the 800-bp *Eco*RI-*Hin*dIII fragment (pSK155 map positions 2.3 to 3.1) (Fig. 4 and 6). Since the well-characterized SEB$^-$ strain RN450 lacks the *entB* gene as well as additional adjacent DNA present in the SEB$^+$ strains, these data suggest that the *entB* gene resides on a discrete genetic element. While the SEB$^-$ strain RN450 lacks the entire "*entB* element," some SEB$^-$ strains contain an incomplete or a related element. These strains lack the *entB* gene but contain the adjacent regions of the element. Two SEB$^+$ strains, DU4916 and 57 dk, have additional *Hin*dIII fragments with homology to the probes flanking the *entB* gene. The region of greatest homology was observed with probes from the region upstream of the *entB* gene (Fig. 2 and 3, and data not shown). Whether this represents an incomplete duplication of the *entB* element or the presence

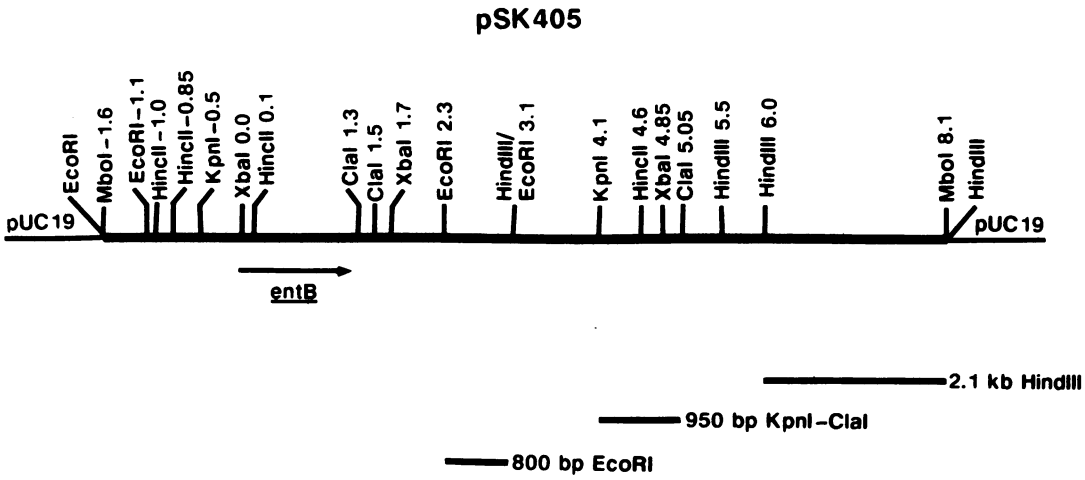

FIG. 7. Restriction map of pSK405. Map position 0.0 corresponds to the start of the *entB* gene. Bars below the map indicate the location of DNA fragments used as probes in this study. The *Mbo*I sites are the result of ligating *Bam*HI and *Bgl*II sites during construction of the plasmid; other *Mbo*I sites have not been mapped. Map coordinates are given in kilobases.

of a related element in the chromosomes of these strains is not known.

Hybridization of the 800-bp *Eco*RI-*Hin*dIII probe from pSK155 to all of the SEB$^-$ strains except U320 (an unrelated strain) suggests that the right-end junction of the *entB* element lies between the *Eco*RI and *Hin*dIII sites defined on the pSK155 map at positions 2.3 and 3.1. Analysis of this region with the 950-bp *Kpn*I-*Cla*I probe from pSK405 revealed that the chromosomes of both SEB$^+$ and SEB$^-$ strains were homogeneous downstream of the *Hin*dIII site identified at position 3.1 on the pSK155 and pSK405 maps (Fig. 9A and B). The region upstream of this *Hin*dIII site was heterogeneous in the SEB$^-$ strains (Fig. 4 and 9B, and data not shown), confirming the location of the right end of the *entB* element.

Analysis of the chromosomal locus of the *entB* element in SEB$^+$ strains with the 800-bp *Eco*RI-*Hin*dIII probe from pSK155 or the 800-bp *Eco*RI probe from pSK405 showed that all the SEB$^+$ strains had identical restriction fragments

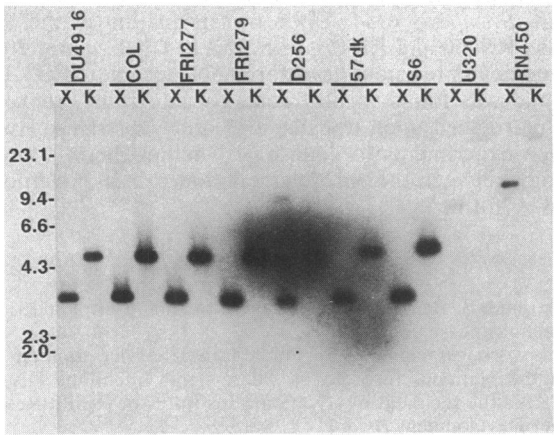

FIG. 8. Southern hybridization analysis of SEB$^+$ *S. aureus* strains with the 800-bp *Eco*RI fragment from pSK405 (map positions 2.3 to 3.1) as the probe. Chromosomal DNA from the indicated SEB$^+$ *S. aureus* strains was digested with *Xba*I (X) or *Kpn*I (K) and analyzed. Bars indicate lambda DNA *Hin*dIII size markers in kilobase pairs.

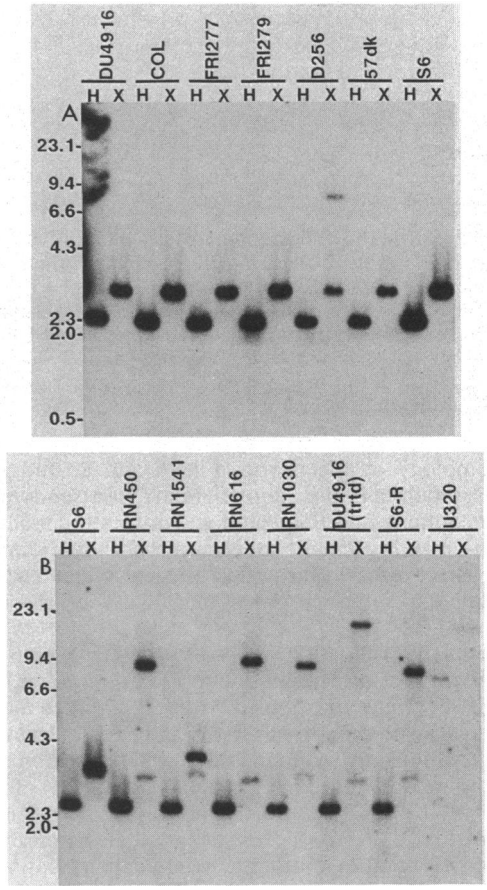

FIG. 9. Southern hybridization analysis of SEB$^+$ and SEB$^-$ *S. aureus* strains with the 950-bp *Kpn*I-*Cla*I fragment from pSK405 (map positions 4.1 to 5.05) as the probe. (A) Analysis of SEB$^+$ strains. Chromosomal DNA from designated strains was digested with *Hin*dIII (H) or *Xba*I (X) and analyzed. (B) Analysis of SEB$^-$ strains. Chromosomal DNA from designated strains was digested with *Hin*dIII (H) or *Xba*I (X) and analyzed. Bars indicate lambda DNA *Hin*dIII size markers in kilobase pairs.

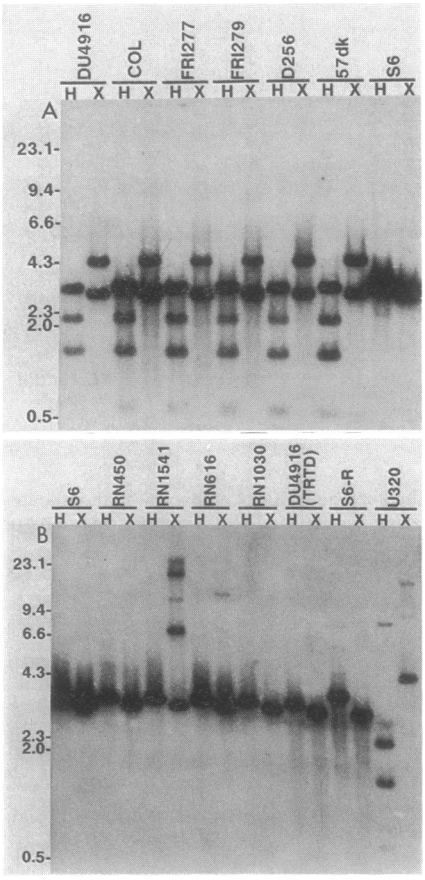

FIG. 10. Southern hybridization analysis of SEB$^+$ and SEB$^-$ *S. aureus* strains with the 2.1-kb *Hin*dIII fragment from pSK405 (map positions 6.0 to 8.1) as the probe. (A) Analysis of SEB$^+$ strains. Chromosomal DNA from designated strains was digested with *Hin*dIII (H) or *Xba*I (X) and analyzed. (B) Analysis of SEB$^-$ strains. Chromosomal DNA from designated strains was digested with *Hin*dIII (H) or *Xba*I (X) and analyzed. Bars indicate lambda *Hin*dIII size markers in kilobase pairs.

with homology to the probe (Fig. 6 and 8, and data not shown). Each of the above restriction endonucleases cleaved within the *entB* element and downstream of the right end of the *entB* element to generate the hybridizing fragments. These data suggest that the *entB* element is positioned at the same chromosomal locus in these SEB$^+$ strains.

Southern hybridization analysis of the region 6 to 8 kb downstream of the *entB* gene with the 2.1-kb *Hin*dIII probe from pSK405 (map positions 6.0 to 8.1) revealed a region of homogeneity common to all SEB$^+$ and SEB$^-$ strains except U320. Additionally, a duplicated or related region that was present in most of the SEB$^+$ strains (except S6) and absent in most of the SEB$^-$ strains (except U320) was identified (Fig. 10A and B). Our studies suggest that the *entB* element is located adjacent to another genetic element that is present in all the SEB$^+$ and most of the SEB$^-$ strains. The observation that this element is lacking in strain U320 while the duplicated region is present suggests that this element may be a member of a family of related genetic elements. One of these related elements was present in the chromosomes of all the SEB$^+$ strains tested except S6 and in the SEB$^-$ strain U320. The role, if any, of the element located adjacent to the *entB* element in SEB genetics is not clear. It is possible that these two elements share homology between their attachment sites, but there is no evidence to support this postulate at present.

Analysis of the chromosomal region upstream of the *entB* gene with the 1.1-kb *Hin*dIII-*Eco*RI fragment from pSK155 (map positions −2.9 to −1.8) as the probe revealed that the SEB$^+$ strains were highly homogeneous in this region of the chromosome (Fig. 5, and data not shown). The largest fragment observed with homology to the probe was the 24-kb *Kpn*I fragment. Chromosomal DNA from the SEB$^-$ strains RN616 and RN1541 showed homology to this probe (Fig. 3), suggesting that these strains contain part of the *entB* element or a related element. Together with the observation that this probe did not hybridize to the SEB$^-$ strain RN450, these data allow the minimum size of the *entB* element to be calculated as 26.8-kb (24-kb *Kpn*I fragment plus the region of pSK155 from −0.5 to 2.3). It is likely that the 24-kb *Kpn*I fragment is internal to the *entB* element since some restriction fragment heterogeneity would be expected between the chromosomes of various SEB$^+$ strains. Extensive efforts to determine more precisely the size of the *entB* element and map the left-end junction by cloning overlapping fragments in *E. coli* have been unsuccessful. The size of the *entB* element rules out the possibility that it is a common transposon. It is possible that the *entB* gene is part of a bacteriophage or a large integrated plasmid. Attempts to induce a putative phage carrying the *entB* gene by UV irradiation have failed to mobilize the toxin gene (6, 9, 26; Johns and Khan, unpublished data). Furthermore, the *entB* marker has only been observed to transfer from one strain to another when a helper phage was used in transduction experiments (9, 27–29). The size of the *entB* element approaches the size of phages, including pS42-D, which carries the gene for staphylococcal enterotoxin A (3, 5, 11). As observed for pS42-D and L54a phages (3, 21), the *entB* element is located at the same chromosomal locus in the SEB$^+$ strains. Additionally, the location of the *entB* gene near the junction of the element agrees with the location of several other toxin genes carried on bacteriophage genomes, such as the diphtheria toxin, streptococcal pyrogenic exotoxin A, and staphylococcal enterotoxin A (3, 16, 20, 33). It is equally possible that the *entB* gene is part of an integrated plasmid, such as pZA10. Altboum et al. (1) reported that a 56.2-kb penicillin resistance plasmid, pZA10, carried the *entB* gene in a clinical strain, *S. aureus* 6344. Upon transformation of the SEB$^-$ strains RN450 and RN451 with pZA10 DNA, about 20% of the penicillin-resistant transformants became SEB$^+$. This plasmid was found to be frequently integrated, reexcised, and rearranged upon transfer to *S. aureus* strains. Hybridization experiments to determine whether there is any homology between the *entB* element and pZA10 have not yet been performed.

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