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Phage-Associated Differences in Staphylococcal Enterotoxin A Gene (*sea*) Expression Correlate with *sea* Allele Class

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Staphylococcus aureus **strains which produced either high or low levels of staphylococcal enterotoxin A (SEA) with a minimal eightfold difference between the two groups were identified. For FRI100 and FRI281A (prototypes for each group), strain differences in the expression of the SEA-encoding gene (*sea*) were found to occur at the level of *sea* mRNA concentration, and part of the difference in expression was associated with the *sea*-containing phages. Southern blot analysis revealed that this phage-associated difference was not due to differences in the copy number of *sea*. Nucleotide sequence analysis of *sea* from FRI281A revealed a new allele of *sea*, with the majority of the sequence differences occurring in the upstream promoter region. Although a strict correlation was observed between the level of SEA production and *sea* allele class for several strains, the sequence differences observed in the upstream region were not sufficient in themselves to alter the expression level of *sea*.**

Staphylococcal enterotoxins are the emetic toxins responsible for the intoxication staphylococcal food poisoning syndrome (4), and they are also virulence factors in some cases of toxic shock-like syndrome (reviewed in reference 5). Currently, the staphylococcal enterotoxins are classified into five major serological groups (SEA through SEE), with division of SEC into three subtypes on the basis of minor antigenic differences. Among the staphylococcal enterotoxins, there is nucleotide sequence identity of 50 to 85% (8).

The gene for SEA (*sea*) is unique from *seb*, *sec*, and *sed* because it is carried by a polymorphic family of lysogenic phages (6). A preferred chromosomal attachment site for the *sea* lysogenic phages between the purine (*pur*) and isoleucine-valine (*ilv*) genes has been found for 24 of the 29 *Staphylococcus aureus* strains examined (6, 25, 31).

Differences in expression occur among the enterotoxin genes with respect to temporal regulation, toxin yield, and regulation by the accessory gene regulator, *agr*. SEA and SED are produced during exponential growth (16, 28), whereas the greatest concentration of SEB and SEC is primarily produced following exponential growth (16, 30). Toxin yield is much lower for strains producing SEA, SED, or SEE (usually less than 8 μg/ml of culture supernatant) compared with strains producing SEB or SEC (often more than 100 μg/ml) (3). Finally, expression of *seb*, *sec*, and *sed* is regulated by *agr* (2, 18, 34), but expression of *sea* is not (39).

For strains containing a particular enterotoxin gene, strain-to-strain variation in the amount of extracellular toxin produced has been observed. For SEB-producing strains, the differences in SEB production were found to be dependent upon host background (12). Different wild-type SEA-producing strains grown under identical culture conditions have also been observed to produce different concentrations of SEA, but the basis for this strain variation was not understood. This study was undertaken to begin to elucidate mechanisms contributing to differences in *sea* expression among wild-type strains.

MATERIALS AND METHODS

Bacterial strains, plasmids, phages, and culture conditions. Bacterial strains, plasmids, and phages used in this study are described in Table 1. SEA-producing *S. aureus* strains were grown in 3% (wt/vol) NZ amine type A (Kraft Inc., Norwich, N.Y.)–1% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.) at 37°C with agitation (200 rpm) in a Controlled Environmental Incubator Shaker (New Brunswick Scientific, Edison, N.J.). Samples for RNA extraction were taken during mid- to late exponential growth at an A_{540} of 1.9 to 2.0. SEA is stable and accumulates during exponential growth of the cultures; little or no additional SEA is produced during stationary growth (16). The ability to detect and accurately quantitate SEA levels by Western blot (immunoblot) analysis was increased by utilizing supernatants from cultures grown for 14 to 16 h.

sea-containing phages from FRI100 and FRI281A (ϕFRI100 and ϕFRI281A) were isolated by UV irradiation of the phage-containing strains, as previously described (6). The phages were used to lysogenize ISP456 (37), generating MJB634 and MJB635, respectively. Genomic DNA from ISP456 does not hybridize to probe A624 (6) or to phage FRI337-1, which contains *sea* (6).

Escherichia coli plasmid DNA was obtained by an alkaline lysis method (1). Restriction enzymes and T4 DNA ligase were from New England BioLabs Inc. (Beverly, Mass.). Alkaline phosphatase was obtained from Boehringer Mannheim (Indianapolis, Ind.). *sea* from strain FRI100, which produces high levels of SEA, was cloned as a 4.8-kb *Sal*I-to-*Eco*RI fragment into pGEM3Zf(+) (Promega Corp., Madison, Wis.), yielding pMJB173. pMJB284 was constructed by insertion of a 1,372-bp *sea*-containing *Eco*RV fragment from pMJB173 into the *Sma*I site of an *E. coli-S. aureus* shuttle plasmid, pCL83 (24).

PCR amplification of FRI281A genomic DNA was used to obtain a 320-bp fragment containing the *sea* upstream promoter region flanked by the naturally occurring *Eco*RV site at the 5′ terminus and by a *Hin*cII site located 45 bp downstream of the translation initiation site. This *Hin*cII site

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TABLE 1. Bacterial strains, plasmids, and phages used in this study

Strain, plasmid, or phage	Relevant characteristic(s)[a]	Source or reference
S. aureus		
Strains		
149	Sea^+	P. Gemski, Walter Reed Army Institute, Washington, D.C.
B2126	Sea^+	P. Gemski
CYL316	Sea^-	C. Y. Lee, Kansas University Medical Center, Kansas City (24)
FRI100	sea_{FRI100}	M. S. Bergdoll, Food Research Institute, Madison, Wis.
FRI281A	$sea_{FRI281A}$	M. S. Bergdoll
FRI337	sea_{FRI337}	M. S. Bergdoll
FRI1104	Sea^+	M. S. Bergdoll
FRI1203	Sea^+	M. S. Bergdoll
FRI1205	Sea^+	M. S. Bergdoll
FRI1222	Sea^+	M. S. Bergdoll
ISP456 (PS 80CR 3)	Sea^-	P. A. Pattee, Iowa State University, Ames (37)
MJB265	sea_{FRI337}	6
MJB634	sea_{FRI100}; ISP456 lysogen of ϕFRI100	This work
MJB635	$sea_{FRI281A}$; ISP456 lysogen of ϕFRI281A	This work
MJB876	$sea_{FRI281A\text{-}FRI100}$; pMJB283 integrated at *geh*	This work
MJB878	sea_{FRI100}; pMJB284 integrated at *geh*	This work
RN450	Sea^-	R. P. Novick, Public Health Research Institute, New York, N.Y. (29)
SA20	Sea^-	S. Iordenescu, Public Health Research Institute, New York, N.Y. (23)
SA92	Sea^+	P. Gemski (26)
Plasmids		
pCL83	Sea^-	C. Y. Lee (24)
pMJB283	pCL83 with a 1.4-kb *Eco*RV insert containing $sea_{FRI281A\text{-}FRI100}$	This work
pMJB284	pCL83 with a 1.4-kb *Eco*RV insert containing sea_{FRI100}	This work
Phages		
ϕFRI100	sea_{FRI100}; *sea*-containing phage originating from FRI100	This work
ϕFRI281A	$sea_{FRI281A}$; *sea*-containing phage originating from FRI281A	This work
E. coli plasmids		
pGEM3Zf(+)	Sea^-	Promega Corp.
pMJB38	sea_{FRI337}	6
pMJB173	pGEM3Zf(+) with 4.8-kb insert containing sea_{FRI100}	This work
pRN6656	Sea^-; pUC18 with 1.2-kb *Hin*cII-*Rsa*I *agr*-containing fragment	R. P. Novick (33)

[a] Subscripts indicate the source of *sea* or type of *sea* allele.

occurs naturally in strain FRI100 but is absent in FRI281A because of a single base substitution. The *Hin*cII site was restored to DNA from FRI281A by PCR using an oligonucleotide primer that replaced the substituted nucleotide. These restriction sites were used to substitute this fragment from $sea_{FRI281A}$ for the analogous *Eco*RV-to-*Hin*cII fragment of sea_{FRI100}, yielding plasmid pMJB283. The *sea* allele in pMJB283 is designated $sea_{FRI281A\text{-}FRI100}$. pMJB283 as well as the isogenic control plasmid, pMJB284, were electroporated as previously described (22) into *S. aureus* CYL316 and integrated into the lipase gene (*geh*) of the chromosome (24).

Western blot analysis. Western blot analysis of SEA from culture supernatants was performed as previously described (22), using antiserum prepared against SEA (obtained from Merlin Bergdoll). Relative concentrations of accumulated SEA were compared by twofold serial dilutions of the supernatants in phosphate-buffered saline (0.2 M sodium phosphate–0.15 M NaCl, pH 7.4). For a given gel, samples indicated as having the same dilution factor contained the same amounts of culture supernatants. Purified SEA was obtained from Toxin Technology (Sarasota, Fla.). Densitometry was used to quantify signal intensity (GS 300 densitometer; Hoefer Scientific Instruments, San Francisco, Calif.). Normal rabbit serum was purchased from Sigma Chemical Co. (St. Louis, Mo.) and was used for identification of non-SEA-related signals.

Labeled probes. The probe used for detection of *sea* mRNA and DNA was derived from pMJB38. It was a 624-bp *Hin*dIII-to-*Bam*HI fragment designated probe A624 that consisted solely of internal *sea* sequence (7). The *agr* probe used was a 1.2-kb *Eco*RI-to-*Bam*HI fragment of pRN6656 (33) that consisted of sequences corresponding to *agrA* and the 3′ end of *agrB* (23). Isolation of DNA fragments from agarose gels and labeling of double-stranded DNA with ^{32}P were performed according to previously described protocols (7).

RNA extraction and Northern (RNA) blot analysis. *S. aureus* total cellular RNA was prepared by the method of Sandler and Weisblum (35). Determination of RNA quality

and concentration and gel electrophoresis and transfer were performed as previously described (34). For a given gel, samples with the same dilution factor contained the same amount of total cellular RNA. The Ambis Radioanalytic Imaging System (AMBIS Systems, San Diego, Calif.) was used to quantify the radioactive signals on the filter, and the relative amount of DNA hybridized to the probe was determined by using data corresponding to sample dilutions that produced signals on the linear portion of the dose (dilution factor)-response (signal intensity) curve.

Copy number determination. For comparisons of copy numbers of *sea* between strains, total genomic DNA was prepared by a previously described method, using lysostaphin (32). The genomic DNA concentration was quantitated by fluorometry (DNA Fluorometer TKO 100; Hoefer Scientific Instruments) with Hoechst dye 33258. A twofold serial dilution of DNA from each sample was prepared; for all samples on a given filter, the initial DNA concentrations were the same. The denatured DNA was transferred to the nitrocellulose filter with a Manifold I Dot Blot apparatus (Schleicher & Schuell, Keene, N.H.), and the filters were hybridized to ^{32}P-labeled probes. For determination of *sea* copy number, probe A624 was used. To verify further that equal concentrations of genomic DNA were applied to the filter, the *agr* probe was hybridized either to filters previously probed with A624 and then stripped by alkaline treatment (6) or to identical filters prepared at the same time with the same dilution series of DNA. *agr* is present in a single copy in the chromosome of *S. aureus* strains (23). The radiation on the filters was quantified by the Ambis Radioanalytic Imaging System.

For comparison of the number of copies of *sea* on *sea*-containing phages, the procedure was similar to that described above, except phage DNA was extracted from samples obtained by UV induction of lysogens (15). To verify that the phage DNA samples were not contaminated with chromosomal DNA, the DNA samples were examined for the inability to hybridize to an *agr* probe.

DNA sequence determination. The nucleotide sequence of sea_{FRI100} was determined by using a recombinant plasmid containing sea_{FRI100}, pMJB173, and the Sequenase kit (United States Biochemical, Cleveland, Ohio). $sea_{FRI281A}$ and DNA upstream of *sea* in strains 149, B2126, FRI1104, FRI1203, FRI1205, FRI1222, and SA92 were sequenced by PCR amplification of genomic DNA followed by direct sequencing of the amplified DNA products as previously described (38), using the Sequenase kit.

Amplification of $sea_{FRI281A}$ was carried out in several rounds, using primers of 18 to 21 nucleotides chosen on the basis of homology to sea_{FRI337} and resulting in amplified products of approximately 300 to 800 bp. The amplified products overlapped, allowing verification of the sequence at all junctions, and both strands of DNA were sequenced. The PCR method used was adapted from a basic protocol previously described (1). The samples containing genomic DNA were denatured at 94°C for 5 min, and then the mixtures went through 30 cycles of denaturation at 92°C, annealing at 45°C, and extension at 72°C. The amplified products were purified from 2% low-melting- and low-gelling-temperature agarose (United States Biochemical), using the Magic PCR Preps DNA Purification System (Promega). The same primers used for PCR amplification were used for sequencing both strands of the DNA.

Nucleotide sequence accession numbers. Nucleotide sequence data have been filed with GenBank. The accession number for *sea* from FRI281A is L22566, and that for the upstream region of *sea* from FRI100 is L22565.

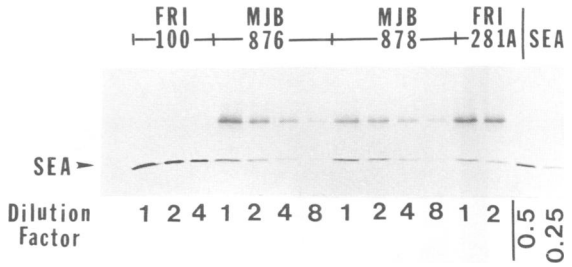

FIG. 1. Western blot analysis with polyclonal antiserum to SEA of culture supernatants (5 μl) from *S. aureus* FRI100, MJB876, MJB878, and FRI281A. The purified SEA on the right represents concentrations of 0.5 and 0.25 μg/ml, as indicated. The arrowhead marks the position corresponding to SEA. Strain designations are shown above and dilution factors are shown below each culture supernatant sample. Samples prepared from different strains with the same dilution factor contain the same amount of culture supernatant. To identify non-SEA-related signals, similar blots reacted with normal rabbit serum showed the same pattern of signals, except the SEA-specific bands were absent (data not shown).

RESULTS

Differences in SEA expression between wild-type strains FRI100 and FRI281A. Western blot analysis using polyclonal antiserum prepared against SEA showed at least an eightfold difference in the amount of accumulated SEA in supernatants from stationary-phase cultures of a strain producing high levels of SEA (FRI100; produces ~4 μg/ml) compared with a strain producing low levels of SEA (FRI281A; produces ~0.5 μg/ml) (Fig. 1). This difference was not the result of a difference in growth rates between the strains; the growth curves were virtually superimposable.

To determine whether differences in *sea* expression between FRI100 and FRI281A were evident at the level of *sea* mRNA, Northern blot analysis was done. Previous analysis of three different Sea$^+$ strains, including FRI100, revealed that the optimal time for detection of *sea* mRNA was when cultures reached an A_{540} of between 1.0 and 2.0, which corresponded to mid- to late exponential growth (11).

Samples of total cellular RNA from FRI100 and FRI281A were prepared from cultures with an A_{540} of 1.9 to 2.0 and compared by Northern blot analysis with probe A624. A signal for *sea* mRNA was evident in a 16-fold dilution of RNA from FRI100 but was only barely visible in the undiluted RNA from FRI281A (Fig. 2). Several independent experiments revealed very low levels of *sea* mRNA from FRI281A compared with FRI100 by Northern analysis.

Phage-associated differences in SEA production. To examine whether there were phage-associated effects on *sea* expression independent of host background, MJB634 and MJB635 were used; these strains were ISP456 derivatives lysogenized with phage φFRI100 and φFRI281A, respectively. In several separate experiments, Western blot analysis with polyclonal antiserum prepared against SEA revealed that supernatants from MJB634 produced three- to fourfold more SEA than MJB635 (Fig. 3).

Determination of relative copy number of *sea*. One possible reason for differences in *sea* expression which would be evident at the level of extracellular SEA and *sea* mRNA concentration could be that the strains contained different copy numbers of *sea* due to either one strain having

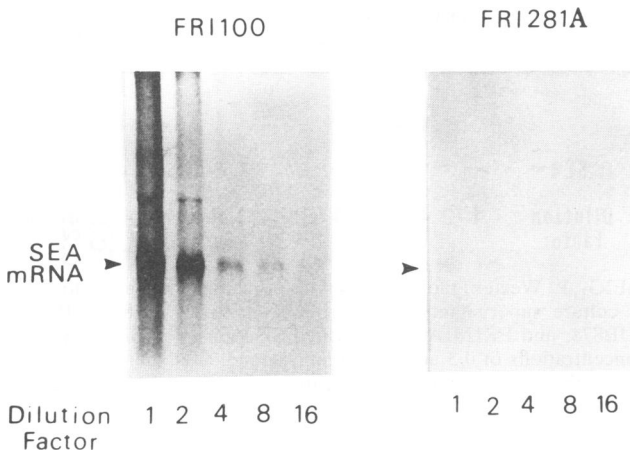

FIG. 2. Northern blot analysis of samples prepared from FRI100 and FRI281A. Samples were collected when the A_{540} of the cultures was 1.9 to 2.0. Total cellular RNA was electrophoresed through a 1% agarose gel, transferred to a Nytran membrane, and reacted with ^{32}P-labeled A624 probe. The arrowhead marks the position corresponding to the major *sea* mRNA species. Strain designations are shown above and dilution factors are shown below each sample. Different samples with the same dilution factor contain the same amount of total cellular RNA. For the undiluted sample from FRI281A, a faint signal was visible on the autoradiogram. Photographs of FRI100 and FRI281A samples are from the same autoradiogram.

more copies of a *sea* phage or different phages having different numbers of *sea*. A dot blot hybridization technique with probe A624 was used to compare *sea* relative copy number for three pairs of samples. Within each pair (wild-type strains FRI100 and FRI281A, phages ϕFRI100 and ϕFRI281A, and lysogens MJB634 and MJB635), the relative copy number of *sea* was the same (data not shown).

Nucleotide sequence comparison of the upstream region for FRI100 and FRI281A. To determine whether the nucleotide sequences in the promoter regions of sea_{FRI100} and $sea_{FRI281A}$ were different and thus a possible cause of a difference in *sea* expression, the nucleotide sequences of the upstream region of *sea* for both strains were analyzed.

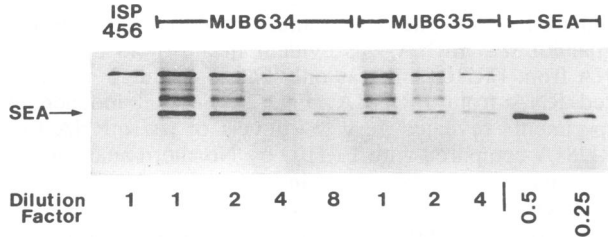

FIG. 3. Western blot analysis with polyclonal antiserum to SEA of culture supernatants (5 μl) from *S. aureus* ISP456, MJB634, and MJB635. The purified SEA on the right represents concentrations of 0.5 and 0.25 μg/ml, as indicated. The arrow marks the position corresponding to SEA. Strain designations are shown above and dilution factors are shown below each culture supernatant sample. Samples prepared from different strains with the same dilution factor contain the same amount of culture supernatant. The control culture, ISP456, does not produce any enterotoxins. To identify non-SEA-related signals, similar blots reacted with normal rabbit serum showed the same pattern of signals, except the SEA-specific bands were absent (data not shown).

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sea_FRI281A     1  gttgtcaataccaagtaagtaagatatctgaaatgtataatagagtaaaa  50
                   ||||| ||||||||||||||||||||||||||||||||||||||||||
sea_FRI100    245  gttgttaataccaagtaagtaagatatctgaaatgtataatagagtaaaa 294

sea_FRI281A    51  atgaaatctttttattatattatagacaagtataaaaaaggtatagtaat 100
                   ||||||||||||     |||||||||||| |||||||| ||||||||||
sea_FRI100    295  atgaaatctttt.....tattatagacaaatataaaaagtgtatagtaat 339

sea_FRI281A   101  atatgtatgtataagtaaataatgataattctataattattgtatataac 150
                   ||||||||||||| ||||| || || ||| ||||||||||||||||||
sea_FRI100    340  atatgtatgtataattaaatgataatcatttcataattattgtatataac 389

sea_FRI281A   151  taataattacttcgacaaaaataatctattatccaaaatattttagataat 200
                   ||| |   | ||||||||
sea_FRI100    390  taaataactacttaacaaaaat.......................... 411
                                                   *
sea_FRI281A   201  aaaaagtttgtatggaattatgctttagaggtgagcaaaatg  242
                              ||||||||||||||||||||||||||||||
sea_FRI100    412  ..............aattatgctttagaggtgagcaaaatg  438
                                        *
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FIG. 4. Comparison of $sea_{FRI281A}$ and sea_{FRI100} upstream nucleotide sequences. The first base pair of sequence determined for both $sea_{FRI281A}$ and sea_{FRI100} was designated 1. The nucleotide sequences were aligned by using the GAP program (University of Wisconsin Genetics Computer Group) set to default parameters (17). The asterisk (*) indicates the translation initiation codon.

Deletion analysis has shown that the 250 bp of DNA upstream of the translation initiation site are sufficient for expression of *sea* (12). The nucleotide sequence of the upstream region of *sea* from FRI100 was determined by using a recombinant plasmid containing sea_{FRI100}, pMJB173. For unknown reasons, we were unable to construct a recombinant plasmid with $sea_{FRI281A}$, so the sequence of $sea_{FRI281A}$ was determined by analysis of PCR-amplified genomic DNA from FRI281A.

A number of sequence differences in the upstream region of *sea* were found for $sea_{FRI281A}$ compared with sea_{FRI100} (Fig. 4). These included 5- and 43-bp insertions in $sea_{FRI281A}$ compared with sea_{FRI100}. The sequence obtained for sea_{FRI100} revealed no differences with respect to the published *sea* sequence, sea_{FRI337} (7), but the sequence of $sea_{FRI281A}$ was unique; therefore, a total of 1.4 kb of sequence was obtained from $sea_{FRI281A}$ to determine the extent of sequence differences throughout the structural gene. Within the coding region of *sea*, four single base pair changes resulted in amino acid substitutions. Two of the changes occurred in the signal sequence but would not be predicted to affect processing or export because they represented changes from one hydrophobic residue to another. These two changes included a C-to-T transition at bp 20 with respect to the published *sea* sequence, which has the first base of the translation initiation site designated 1 (6), resulting in an amino acid substitution from Thr to Ile (these changes are designated as C→T [20], Thr→Ile). The second change was T→G [47] (Leu→Trp). The other two amino acid substitutions occurred in the first third of the protein (A→G [133], Thr→Ala, and G→A [250], Asp→Asn). There were also five silent base pair changes (T→C [22], G→A [258], G→A [333], T→C [390], and T→A [685]).

Examination of *sea* upstream sequences and relative SEA expression levels for additional strains. Ten SEA-producing strains, including FRI100 and FRI281A, were examined by Western blot analysis with polyclonal antiserum to SEA (data not shown). On the basis of the amount of SEA produced, the strains could be divided into two classes. FRI1104, MJB265, and SA92 produced about as much SEA as FRI100, whereas strains FRI1203, FRI1205, FRI1222, 149, and B2126 produced as much as or less SEA than FRI281A (Table 2). Nucleotide sequence analysis of the region, including at least 250 bp upstream of the translation initiation site, also revealed two distinct classes of *sea* alleles (Table 2). All strains which produced high levels of SEA had an upstream region identical to that of sea_{FRI100} (*sea* allele

TABLE 2. Summary of *sea* allele class for *S. aureus* strains

Strain	*sea*		Expression level[a]
	Region sequenced	Allele class	
FRI337	1–1693[b]	1[c]	High
FRI100	245–490	1	High
FRI1104	245–440	1	High
SA92	235–509	1	High
FRI281A	1–1421[d]	2[e]	Low
FRI1203	11–297	2	Low
FRI1205	31–253	2	Low
FRI1222	2–315	2	Low
B2126	59–250	2	Low
149	4–255	2	Low

[a] The relative level of expression was determined by Western immunoblot with polyclonal antiserum to SEA (data not shown). There is at least an eightfold difference between high and low expression levels.

[b] The region sequenced for all strains with *sea* allele class 1 is in relationship to the sequence for sea_{FRI100}, which has the translation start site at nucleotide 436.

[c] Alleles designated class 1 have upstream nucleotide sequences identical to that of sea_{FRI100}.

[d] The region sequenced for all strains with *sea* allele class 2 is in relationship to the sequence for $sea_{FRI281A}$, which has the translation start site at nucleotide 240.

[e] Alleles designated class 2 have upstream nucleotide sequences identical to that of $sea_{FRI281A}$.

class 1), and all strains which produced low levels of SEA had an upstream region identical to that of $sea_{FRI281A}$ (*sea* allele class 2).

Effect of the upstream promoter region on SEA expression. To determine whether the sequence differences in the upstream regions of $sea_{FRI281A}$ and sea_{FRI100} contributed to differences in *sea* expression, SEA production by MJB876 and MJB878 (CYL316 derivatives with $sea_{FRI281A\text{-}FRI100}$ from pMJB283 and sea_{FRI100} from pMJB284 integrated into *geh*, respectively) was compared. In several independent experiments, Western blot analyses with polyclonal antiserum prepared against SEA revealed no difference in SEA production between MJB876 and MJB878 (Fig. 1). There was also no difference in SEA production between $sea_{FRI281A\text{-}FRI100}$ and sea_{FRI100} in a different host background strain, ISP456 (data not shown).

DISCUSSION

Two wild-type *S. aureus* strains and their *sea*-containing phages were examined to begin to identify factors that account for differences in *sea* expression. FRI100 produced eightfold more SEA than FRI281A (Fig. 1). This difference in *sea* expression was evident at the level of *sea* mRNA concentration (Fig. 2), and part of the difference in *sea* expression was associated with the different *sea*-containing phages. The ISP456 lysogen containing phage ϕFRI100 (MJB634) produced three- to fourfold more SEA than the lysogen containing phage ϕFRI281A (MJB635) (Fig. 3). It was not possible to directly compare SEA production between the lysogens and their wild-type parents because of differences in their growth patterns. This phage-associated difference in *sea* expression was not due to a difference in the number of *sea* copies per phage. Phage mediation of differences in SEA expression was not unexpected given the fact that the *sea*-containing phages are not identical and exhibit restriction fragment length polymorphisms (6). The observation that the eightfold difference seen in the wild-type strains was not completely accounted for by the phages suggested that some (about 50%) of the difference was dependent upon host strain background. For *S. aureus* SEB-producing strains, differences in SEB expression have so far only been found to be strain dependent (13).

Differences in *sea* expression between FRI100 and FRI281A and between MJB634 and MJB635 were not due to differences in *sea* copy numbers. Other experiments in which a single copy of *sea* was introduced into the *S. aureus* chromosome have been used to normalize the dot blot analyses and have indicated that the actual copy number for *sea* in FRI100, FRI281A, MJB634, and MJB635 was equal to 1 (data not shown).

Nucleotide sequence analysis of the 250 bp upstream of the translation initiation site for sea_{FRI100} and $sea_{FRI281A}$ revealed a large number of sequence differences (Fig. 4). Mutational and deletion analyses of DNA upstream from the base pair corresponding to the 5′ end of *sea* mRNA have been used to localize the putative promoter region for sea_{FRI337} (12). On the basis of this analysis, the putative −35 and −10 regions of the promoter were identical for sea_{FRI100} and $sea_{FRI281A}$.

The remainder of *sea* from FRI281A was sequenced and compared with *sea* from FRI337 to determine the extent of differences throughout *sea* sequences. $sea_{FRI281A}$ was found to be a distinct allele of *sea* on the basis of four predicted amino acid changes. A comparison of sea_{FRI337} and $sea_{FRI281A}$ revealed 98.8% nucleotide sequence identity and derived amino acid sequence identities of 99.1 and 98.4% for the mature protein and the precursor form, respectively. Variant alleles have been reported for other related toxin genes, including *sec* (9, 10, 14, 21), the gene encoding streptococcal pyrogenic exotoxin type A (*speA*) (27), and the gene encoding toxic shock syndrome toxin (*tsst*) (19, 20).

Examination of a total of 10 Sea^+ strains revealed two classes on the basis of either high or low levels of SEA expression. There was at least an eightfold difference between high- and low-level production of SEA among the strains. Sequence analysis of the upstream region showed that all strains producing high levels of SEA had an upstream region identical in sequence to sea_{FRI100}, whereas all strains producing low levels of SEA had an upstream sequence identical to that of $sea_{FRI281A}$. Surprisingly, the sequence differences in the upstream region of $sea_{FRI281A}$ were not sufficient in themselves to cause the decreased expression level of SEA.

The strict correlation observed between upstream sequence differences and SEA expression level (Table 2) is consistent with the interpretation that the *sea* allele class is a good indicator of the phage on which *sea* resides (and thus its level of SEA expression), but the sequence differences observed in the upstream region of *sea* are not solely responsible for the differences observed in SEA expression. It is possible that information encoded elsewhere on the phage interacts with these regions of sequence differences, resulting in higher or lower expression of *sea*. It is also possible that a phage-dependent mechanism completely independent of differences in the *sea* promoter region is responsible for variation in *sea* expression between the lysogens and wild-type strains.

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