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Identification of a Chromosomal Determinant of Alpha-Toxin Production in *Staphylococcus aureus*

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Production of α-toxin (the Hla$^+$ phenotype, controlled by the *hla*$^+$ gene and scored as α-hemolytic activity) is a property of some isolates of *Staphylococcus aureus* NCTC 8325 and not of others. Genetic transformation between strains differing in the Hla phenotype revealed that the *hla*$^+$ gene resides in the following sequence: *purB110-bla*$^+$*-hla*$^+$*-ilv-129-pig-131*; previously, the enterotoxin A (*entA*) gene of strain S-6 was shown to map very close to *hla*$^+$. The *hla* mutations occurring naturally in strain Ps6 and after various mutagenic treatments in strains 8325 and 233 also mapped between *bla*$^+$ and *ilv-129*. Among the isolates of strain 8325, the Hla$^+$ phenotype was always associated with fibrinolytic activity, whereas Hla$^-$ isolates were non-fibrinolytic. This relationship was also observed among transformants selected for their Hla$^+$ or Hla$^-$ phenotypes. The failure of Hla$^-$ strains and mutants to revert to Hla$^+$ at detectable frequencies, the instability of the Hla$^+$ phenotype, and the previously observed pattern of recombination of the *hla*$^+$ and *entA*$^+$ determinants lend support to the view that *hla*$^+$ may reside on a transposon; according to this view, Hla$^-$ mutants have lost the *hla*$^+$-bearing transposon. It remains unclear whether *hla*$^+$ is the structural gene for α-toxin.

Among the extracellular proteins produced by *Staphylococcus aureus* that contribute to pathogenicity, α-toxin is thought to be of major significance. The α-toxin is most often and easily detected by its hemolytic activity toward several species of erythrocytes, and hence it is often referred to as the α-hemolysin. However, it also is cytocidal and cytolytic toward many cell types, exhibits both dermonecrotic and neurotoxic activities, and is lethal to a variety of experimental animals (17). This diversity of toxic activities has led to proposals that α-toxin is better referred to as a cytolytic toxin (1) or a membrane-damaging toxin (6). The term α-toxin is used throughout this report.

Considerable interest has been directed toward an understanding of the genetic control of α-toxin. Not all strains of *S. aureus* produce α-toxin, and the stability of α-toxin activity among those strains that do varies (3). Blair and Carr (2) demonstrated that the ability to elaborate α-toxin was often acquired by a nontoxigenic strain after lysogenization by a phage derived from a toxin-producing strain. Hendricks and Altenbern (5) examined 26 strains, including strain 233 (see below), for evidence of involvement of prophage in α-toxin production; no support for this hypothesis was obtained. In a study of *S. aureus* isolates from human pus, Witte (20) concluded that α-toxin activity was controlled by a plasmid is some strains. McClatchy and Rosenblum (7, 8) reported the isolation and a transduction analysis of many nitrous acid- and ultraviolet-induced nonhemolytic mutants of *S. aureus* strain 233. They concluded that two groups of nonhemolytic mutants existed. The mutants in group I were thought to affect only the structural gene of α-toxin, whereas the mutants in group II appeared to be regulatory mutants that were deficient in the production of both α-toxin and fibrinolysin. Recombination (measured by generalized transduction) was observed at significant frequencies only between mutants in different groups. Wheeler (19) also observed a relationship between α-toxin and fibrinolysin production; survivors of ultraviolet radiation often were negative for both activities.

We observed that α-toxin activities varied among many multiply marked derivatives of *S. aureus* strain 8325. This strain is devoid of known plasmids (10), is amenable to genetic analysis by transformation and transduction, and provides the basis for our current knowledge of the organization of the chromosome in lytic group III staphylococci (13, 14). Therefore, this study was undertaken to identify the chromosomal locus responsible for α-toxin production in *S. aureus*.

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MATERIALS AND METHODS

Bacteria. The strains of *S. aureus* used in this study, their genotypes, and their origins are listed in Table 1.

Culture media. In addition to commercially available dehydrated culture media, various formulations of a complete defined synthetic agar were used (14). Appropriate additions and deletions were made to the complete defined synthetic agar to prepare media for the selection and scoring of various markers. All culture media contained thymine (20 μg/ml) and adenine, guanine, cytosine, and uracil (each at 5 μg/ml) unless their omission was specifically required for selective purposes. Fibrinolysin production was assayed by a modification of the fibrin agar method of Vogelsang et al. (18). Sterile porcine plasma (12%, vol/vol) was added to sterile cooled Trypticase soy agar, which was then held at 56°C for 20 min before pouring. After drying overnight, the agar was surface inoculated with the isolates to be scored. Fibrinolytic activity, as revealed by the clearing of fibrin in the area adjacent to growth, was scored after incubation at 35°C for 48 h.

Transformations. The procedures used in transformation have been described previously (12–14). The genetic markers used, their phenotypes, and the compositions of the media used for their selection and scoring are summarized in Table 2. The cotransfer frequencies by transformation of two markers, A and B, are expressed cotransfer frequencies (EC), in which EC is the frequency of AB/frequency of A or the frequency of AB/frequency of B. Map distances between markers are expressed as 1 − EC. The ability to elaborate α-toxin was scored by measuring α-hemolytic activity (the Hla$^+$ phenotype [Table 2]) on sheep blood agar. The Hla phenotype was scored among transformants as an unselected marker by picking these transformants from the selective medium to sheep blood agar. The rationale for this procedure, which was required for accurate scoring of the Hla phenotype, has been given by Pattee and Glatz (13). Although far more sensitive than sheep blood to α-toxin, rabbit blood was not used because this greater sensitivity resulted in excessively large zones of hemolysis, which interfered with the scoring of the Hla phenotype among modestly large populations of recombinants on individual plates. The identity of the α-toxin was confirmed in this study by the inhibition of α-hemolytic activity by β-toxin on sheep blood agar (4), the greater sensitivity to hemolysis exhibited by rabbit erythrocytes relative to sheep erythrocytes, and the total inhibition of α-hemolysis on rabbit blood agar by specific anti-staphylococcal α-hemolysin antisera (Burroughs Wellcome Co., Research Triangle Park, N.C.). The δ-toxin was differentiated from the α-toxin by its insensitivity to antisera specific for the α-toxin and by its potentiation by β-hemolysin (4). The β-toxin was identified by its incomplete hemolytic activity on sheep blood agar plates.

TABLE 1. *Designations, genotypes, and origins of S. aureus strains*

Stock no.	Genotype[a]	Origin and/or reference(s)
ISP1	8325 *thy-101 pig-131*	14
ISP2	8325 *nov-142 pig-131 hla-156*	14
ISP5	8325 *thy-101 thrB106 ilv-129 pig-131*	14
ISP44	8325 *thy-101 purB110 pig-131*	14
ISP92	Ps6 *tmn-3106*	12
ISP94	Ps6 *tmn-3511 hla-295*	12
ISP99	8325 *thy-101 purB110 tmn-3106 pig-131* φ11[a]	ISP44 × ISP92 DNA[b]
ISP103	8325 *thy-101 thrB106 ilv-129 pig-131 tmn-3106*	ISP5 × ISP92 DNA
ISP161	8325 *nov-142 tmn-3106 pig-131*	13
ISP163	8325 *nov-142 tmn-3106 bla$^+$ pig-131 hla-156*	13
ISP283	8325-4 Ω5[Chr::Tn*551*] *tmn-3106 nov-142 pig-131 hla-156*	RN496 × φ80α/ISP161 twice[c]
ISP433	8325 *nov-142 pig-131 hla-156 tmn-3106 purB110 φ11*[a]	ISP2 × ISP99 DNA
ISP436	8325 *nov-142 pig-131 hla-156 tmn-3106 purB110* Ω7[Chr::Tn*551*]	ISP433 × φ80α/RN497
ISP446	8325 *thy-101 thrB106 ilv-219 pig-131 purB110* Ω7[Chr::Tn*551*]	ISP5 × ISP436 DNA
ISP448	8325 *thy-101 thrB106 ilv-129 pig-131 purB110 tmn-3106*	ISP446 × ISP103 DNA
ISP461	233 *hlaB151* (formerly 152a)	7, 8; Rosenblum[d]
ISP463	233 *hlaA153* (formerly 152i)	7, 8; Rosenblum
ISP465	233	7, 8; Rosenblum
ISP484	8325 *thy-101 thrB106 ilv-129 tmn-3106 purB110 pig-131* Ω40[Chr::Tn*551*]	13
RN496	8325-4 Ω5[Chr::Tn*551*] *pig-131 hla-156*	9; Novick[e]
RN497	8325-4 Ω7[Chr::Tn*551*] *pig-131*	9; Novick

[a] Several of these strains carry Tn*551*(Em), a transposon conferring resistance to erythromycin (11, 15), at specific chromosomal sites. Each site bears a different insertion number, which is preceded by the symbol Ω.

[b] ISP44 transformed with DNA prepared from ISP92.

[c] RN496 transduced with phage 80α propagated on ISP161.

[d] E. D. Rosenblum, University of Texas Health Science Center, Dallas.

[e] Richard P. Novick, Department of Plasmid Biology, The Public Health Research Institute of the City of New York, Inc., New York, N.Y.

TABLE 2. *Genetic markers, their phenotypes, and the compositions of the media used for their selection and scoring*

Marker	Phenotype of marker	Basal agar[a]	Modification of basal agar for selection and scoring
purB110	Pur$^-$ (adenine and guanine requirement)	CDS	Omission of adenine and guanine
ilv-129	Ilv$^-$ (requirement for L-isoleucine and L-valine)	CDS	Omission of isoleucine and leucine; valine reduced to 20 μg/ml; 1% (wt/vol) sodium pyruvate added
tmn-3106	Tmn^r (resistance to tetracycline and minocycline)	CDS	Addition of either 1 μg (for selection) or 3 μg (for scoring) of tetracycline per ml
bla$^+$	Pc^r (resistance to penicillin by β-lactamase production)	CDS, TSA, or BHI	Addition of 0.3% soluble starch and 7.25 μM CBAP; flood with penicillin–I_2 solution (15)[b]
hla-151, hla-153, hla-156, hla-162, hla-295	Hla$^-$ (failure to elaborate α-hemolysin)	TSA	Addition of 5% (vol/vol) citrated sheep blood
Ω1[Chr::Tn*551*], Ω7[Chr::Tn*551*][c]	Em^r (resistance to erythromycin)	CDS or BHI	Addition of 1 μg (for selection) or 10 μg (for scoring) of erythromycin per ml

[a] CDS, Complete defined synthetic agar; TSA, Trypticase soy agar; BHI, brain heart infusion agar.
[b] CBAP, 2-(2′-carboxyphenyl)-benzoyl-6-amino-penicillanic acid (Sigma Chemical Co., St. Louis, MO).
[c] Specific chromosomal insertions of Tn*551*(Em), a transposon originating from the pI258 plasmid and conferring constitutive resistance to erythromycin (11, 25).

RESULTS

Instability of HlA$^+$. The capacity of strain 8325 to elaborate α-toxin is unstable, as reflected by the observation that strains ISP1 and ISP5 are strongly Hla$^+$, whereas strain ISP2 is Hla$^-$. Among 44 other derivatives of strain 8325 in our stock collection that were examined for α-toxin activity, 19 were Hla$^-$; the loss of α-toxin activity in these derivatives could not always be traced to a parent strain used in strain construction, a mutagenic treatment, or any other specific event. In every instance examined, the Hla$^+$ derivatives of strain 8325 were fibrinolytic, whereas Hla$^-$ derivatives were devoid of detectable fibrinolytic activity. The β- and δ-toxin activities of these derivatives also varied, but independently of one another or α-toxin.

***hla-156* marker mapped by transformation.** To attempt the identification of the site occupied by the *hla*$^+$ marker, strain ISP2 was used as the recipient in a series of transformations. Strain ISP2 is Hla$^-$, a characteristic attributed to a spontaneous *hla-156* mutation, and is also Tmn^s and Em^s. When the deoxyribonucleic acids (DNAs) from several Hla$^+$ strains were used to transform ISP2, the *hla*$^+$ marker appeared to be to the right of *tmn-3106* in linkage group III; this region consists of the following sequence: *tmn*-Ω1[Chr::Tn*551*]-*purB110*-*bla*$^+$-*ilv-pig* (13).

To analyze further the position of *hla-156* relative to *purB110* and *ilv-129*, strain ISP446 (Em^r Pur$^-$ Hla$^+$ Ilv$^-$) was transformed with strain ISP2 DNA (Em^s Pur$^+$ Hla$^-$ Ilv$^+$). The Em^r marker of ISP446 has been attributed to Ω7[Chr::Tn*551*], which is probably allelic with Ω1[Chr::Tn*551*] (unpublished data). Selection was made for Pur$^+$ and Ilv$^+$ transformants, which were then analyzed for the unselected markers. When the Pur$^+$ transformants were examined, the donor Em^s and Hla$^-$ phenotypes segregated independently, placing *hla-156* between *purB110* and *ilv-129*. This conclusion was confirmed by the fact that only about 50% of the transformants selected for either Pur$^+$ or Ilv$^+$ were Hla$^-$, whereas between 96 and 97% of the transformants that had acquired both Pur$^+$ and Ilv$^+$ were Hla$^-$ (data not shown).

The *bla*$^+$ marker is known to be between *purB110* and *ilv-129* (15), and therefore it was necessary to determine the position of *hla-156* relative to *bla*$^+$. ISP446 (Em^r Pur$^-$ Pc^s Hla$^+$ Ilv$^-$) was transformed with ISP163 DNA (Em^s Pur$^+$ Pc^r Hla$^-$ Ilv$^+$); selection was for Pur$^+$, Ilv$^+$, and Pur$^+$ Ilv$^+$, and these classes of transformants were then analyzed for all unselected markers involved in this cross (Table 3). An analysis of 746 Pur$^+$ transformants placed *bla*$^+$ between *purB110* and *hla-156* because all of the Pur$^+$ Hla$^-$ transformants (Table 3, classes E, F, G, and H) were also Pc^r, whereas the Pur$^+$ Hla$^+$ transformants were either Pc^s (classes A and B) or Pc^r (classes C and D). The analysis of 708 Ilv$^+$ transformants confirmed this conclusion; Ilv$^+$ Hla$^-$ transformants were either Pc^r or Pc^s, whereas almost all of the Ilv$^+$ Pc^r transformants were Hla$^-$. The exceptions (classes L, M, and N)

TABLE 3. *Distribution of phenotypes among purine-independent (Pur$^+$) and isoleucine-valine-independent (Ilv$^+$) transformants recovered after the transformation of strain ISP446 (Em^r Pur$^-$ Pc^s Hla$^+$ Ilv$^-$) with DNA from strain ISP163 (Em^s Pur$^+$ Pc^r Hla$^-$ Ilv$^+$)*

Phenotype	Presence in class:													% of recombinants detected in class:[a]											Frequency of:[b]	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Reversion	Transformation
Em	r^c	s^d	r^c	s^d	r^c	s^d	r^c	s^d	r^c	r^c	r^c	s^d	s^d	s^d																
Pur	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$	$-^c$	$-^c$	$-^c$	$+^d$	$+^d$	$-^c$																
Pc	s^c	s^c	r^d	r^d	r^d	r^d	r^d	r^d	s^c	s^c	r^d	r^d	s^c	s^c																
Hla	$+^c$	$+^c$	$+^c$	$+^c$	$-^d$	$-^d$	$-^d$	$-^d$	$+^c$	$-^d$	$-^d$	$+^c$	$+^c$	$-^d$																
Ilv	$-^c$	$-^c$	$-^c$	$-^c$	$-^c$	$-^c$	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$	$+^d$																
Pur$^+$ (746)[c]															6.8	4.0	3.2	9.3	8.8	30.4	9.4	27.4				0.5	0.1		<5	2,165
Ilv$^+$ (708)																					3.1	7.8	33.5	51.3	4.1	0.1		0.1	<5	2,591

[a] Percentages of all of the recombinants examined.

[b] Expressed as number of transformants recovered per 10^9 colony-forming units. We used excess DNA and 9.2×10^8 colony-forming units per ml of transformation suspension.

[c] Recipient phenotype.

[d] Donor phenotype.

[e] The numbers in parentheses are the numbers of transformants analyzed for unselected phenotypes.

were attributed to rare quadruple crossovers. The results also clearly demonstrate that Ω7[Chr::Tn*551*] is to the left of *purB110*, whereas *bla*$^+$ and *hla-156* are between *purB110* and *ilv-129*. The linkage relationship of the Ω7-[Chr::Tn*551*] element and the *purB110*, *bla*$^+$, *hla-156*, and *ilv-129* markers in strain 8325 is shown in Fig. 1. The distances between the markers are expressed as 1 − EC, as calculated from the distribution of unselected markers among the transformants shown in Table 3.

Relationship of *hla-156* to other *hla* mutational sites. In view of the transduction analysis of the *hla*$^+$ gene by McClatchy and Rosenblum (8), it was important to relate the spontaneously occurring *hla-156* mutational lesion to the *hla* mutations induced with HNO_2 and ultraviolet radiation. DNAs from representative Hla$^-$ mutants of strain 233 (strains ISP461 and ISP463 [Table 1]; both Pur$^+$ Hla$^-$ Ilv$^+$ Pig$^+$) were used to transform strain ISP448 (Pur$^-$ Hla$^+$ Ilv$^-$ Pig$^-$); selection was for Pur$^+$ and Ilv$^+$. Because the results with these two DNAs were essentially identical, only those for ISP461 are presented (Table 4 and Fig. 2). Although the *hla-151* and *hla-153* sites were indistinguishable and occupied the same region between *purB110* and *ilv-129* occupied by *hla-156*, it is not certain from these data whether all three *hla* mutational sites are identical.

Five additional *hla* mutations were isolated from ISP1 after it was treated with *N'*-methyl-*N'*-nitro-*N*-nitrosoguanidine (21). DNAs were prepared from these Hla$^-$ mutants and were used to transform ISP484 (Pur$^-$ Hla$^+$ Ilv$^-$). A total of 20 Pur$^+$ Ilv$^+$ transformants were isolated, purified from each transformation, and scored for Hla. In every instance, all of these Pur$^+$ Ilv$^+$ transformants were Hla$^-$. These Hla$^-$ mutants of ISP1, like all of the other Hla$^-$ mutants examined in this study, were never observed to give rise to Hla$^+$ revertants. Thus, it appears that the phenotypic change from Hla$^+$ to Hla$^-$ occurs readily (either spontaneously or in response to ultraviolet radiation, HNO_2, or nitrosoguanidine), whereas the reverse change does not occur.

Association of Hla and fibrinolysin production. Because McClatchy and Rosenblum (7, 8) reported a correlation between α-toxin production and fibrinolytic activity in strain 233 and in the Hla$^-$ mutants isolated from this strain, this relationship was examined in the strains and recombinants involved in the experiments described in this report. In contrast to the findings of McClatchy and Rosenblum, we were not able to detect fibrinolytic activity in their group I (ISP463; =152i [7]) or group II

(ISP461; =152a [7]) Hla$^-$ mutants or in parent strain 233 (ISP465). As previously noted, however, derivatives of strain 8325 that were Hla$^+$ were always fibrinolytic, whereas Hla$^-$ derivatives of strain 8325 were always devoid of detectable fibrinolytic activity. In addition, the relationship between Hla$^+$ and fibrinolytic activity was apparent among recombinants. Table 5 summarizes the α-toxin and fibrinolytic activities of recombinants recovered from three transformations involving the fibrinolytic strain ISP448 (Pur$^-$ Hla$^+$ Ilv$^-$) and the DNAs from three nonfibrinolytic donors (all Pur$^+$ Hla$^-$ Ilv$^+$). A nearly perfect correlation between cotransformation of the Hla$^-$ and non-fibrinolytic phenotypes of the donors was observed. These results also showed that *hla-162* (a presumed spontaneous *hla* mutation in strain Ps6) is probably allelic with the other *hla* mutations already mapped between *purB110 and ilv-129*.

DISCUSSION

The results of this study confirm those of a previous study (13), namely, that the *hla*$^+$ gene of *S. aureus* is located on the chromosome between *bla* and *ilv-129*. Although these results were obtained largely with derivatives of strain 8325, there is evidence that strains 233 and Ps6 also carry an *hla*$^+$ marker (or a spontaneous *hla* mutation) in this same region. Thus, it appears that at least the lytic group III strains share a common organization of this region of the chromsome. However, there are two major unanswered questions concerning the *hla*$^+$ determinant. First, the correlations observed by us and others between α-toxin and fibrinolysin production raise the possibility that, rather than being the structural gene for the α-toxin, the *hla*$^+$ gene plays an undefined role in the regulation of several extracellular proteins, of which α-toxin is but one. The isolation of mutants that elaborate a temperature-sensitive α-toxin or fibrinolysin and a definition of the chromosomal site responsible for such a mutation would provide the means for clarifying this situation.

The other unanswered question concerns the unstable nature of the Hla$^+$ phenotype. It seems that if the *hla*$^+$ marker is a conventional chromosomal gene, then it exhibits an unexpectedly high mutability that also affects fibrinolysin pro-

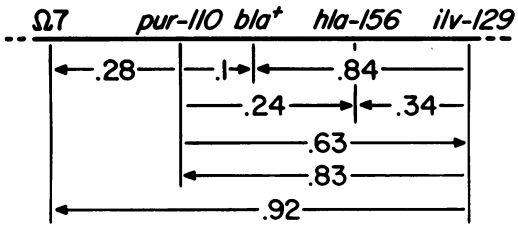

FIG. 1. *Linkage relationships among the Ω7[Chr::Tn551], purB110, bla*$^+$*, hla-156, and ilv-129 markers. Numerical values are expressed as 1 − EC as calculated from the analysis of unselected markers shown in Table 3. The arrows point from selected to unselected markers.*

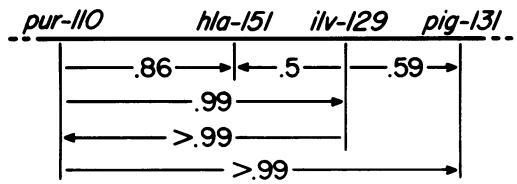

FIG. 2. *Linkage relationships among the purB110, hla-151, ilv-129, and pig-131 markers. Numerical values are expressed as 1 − EC, as calculated from the analysis of unselected markers shown in Table 4. The arrows point from selected to unselected markers.*

TABLE 4. *Distribution of phenotypes among purine-independent (Pur*$^+$*) and isoleucine-valine-independent (Ilv*$^+$*) transformants recovered after the transformation of strain ISP448 (Pur*$^-$ *Hla*$^+$ *Ilv*$^-$ *Pig*$^-$*) with DNA from strain ISP461 (Pur*$^+$ *Hla*$^-$ *Ilv*$^+$ *Pig*$^+$*)*

Phenotype	Presence in class:								% of recombinants detected in class:[a]								Frequency of:[b]	
	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H	Reversion	Transformation
Pur	+[c]	+[c]	+[c]	+[c]	−[d]	−[d]	−[d]	−[d]										
Hla	+[d]	−[c]	−[c]	−[c]	+[d]	−[c]	+[d]	−[c]										
Ilv	−[d]	−[d]	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]										
Pig	−[d]	−[d]	−[d]	+[c]	−[d]	−[d]	+[c]	+[c]										
Pur$^+$ (186)[e]									86.1	13.4	0.5	0.0					<5	367
Ilv$^+$ (221)											0.0	0.0	29.9	29.0	19.9	21.3	<5	1,729

[a] Percentages of all the recombinants examined.
[b] Expressed as number of transformants recovered per 10^9 colony-forming units. We used excess DNA and 8.5 × 10^8 colony-forming units per ml of transformation suspension.
[c] Donor phenotype.
[d] Recipient phenotype.
[e] The numbers in parentheses are the numbers of transformants analyzed for unselected phenotypes.

TABLE 5. *Correlation of the α-hemolytic and fibrinolytic activities of recombinants isolated from strain ISP448 (Pur⁻ Hla⁺ Ilv⁻; fibrinolytic) after transformation with DNAs from three Pur⁺ Hla⁻ Ilv⁺ non-fibrinolytic strains*

Donor strain	Phenotype of transformant	No. fibrinolysin positive	No. fibrinolysin negative
ISP2	Pur⁺ Hla⁺ Ilv⁻	58	2
	Pur⁻ Hla⁺ Ilv⁺	48	0
	Pur⁺ Hla⁻ Ilv⁺	0	50
ISP94	Pur⁺ Hla⁺ Ilv⁻	23	0
	Pur⁺ Hla⁻ Ilv⁻	0	45
ISP283	Pur⁺ Hla⁺ Ilv⁻	57	1
	Pur⁺ Hla⁻ Ilv⁻	0	20

duction. The region of the chromosome occupied by *hla*⁺ is shared by the determinant of enterotoxin A production (the *entA*⁺ gene [13]). In the study of Pattee and Glatz (13) the order of the *entA*⁺ and *hla*⁺ markers relative to *bla*⁺ was analyzed by multifactorial crosses by transformation. The *hla*⁺ and *entA*⁺ markers were clearly between *bla*⁺ and *ilv*⁺; however, because of minor classes of recombinants that were either the result of quadruple crossover events or a spontaneous loss of either *hla*⁺ or *entA*⁺ or both, the order of *hla*⁺ and *entA*⁺ relative to *bla*⁺ could not be determined. These results led to the suggestion that either *hla*⁺ or *entA*⁺ or both were on transposable genetic elements. This proposal, which was also made by Rogolsky (17), offers a tenable explanation for the observations that an unexpectedly high number of derivatives of strain 8325 are Hla⁻ and that the Hla⁻ derivatives and mutants described in this study did not revert to Hla⁺. Such an element, which may or may not be site specific (16), would also provide a framework to explain the earlier observations that α-toxin production is under plasmid control in some strains (20) and is involved in phage conversion in others (2).

Depending on the strain examined, the region of the chromosome involved in this study can include determinants for production of β-lactamase (*bla*⁺), α-toxin (*hla*⁺), and enterotoxin A (*entA*⁺). The *hla*⁺ marker also appears to influence fibrinolytic activity. It has been suggested that all of these elements reside on transposons, a suggestion for which no definitive evidence is available. However, this region of the chromosome appears to be of major importance in the production of clinically important extracellular products and to be amenable to further detailed genetic analysis. Our failure to distinguish readily between the two classes of *hla* mutations described by McClatchy and Rosenblum (7, 8) by using transformation suggests that generalized transduction would be the method of choice in gaining a greater knowledge of these elements and their interrelationships.

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