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Transduction of Staphylococcal Enterotoxin B Synthesis: Establishment of the Toxin Gene in a Recombination-Deficient Mutant†

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Cotransduction of enterotoxin B synthesis into a recombination deficient mutant only occurred when the donor contained the pEntB plasmid. Enterotoxin B from chromosomal genotypes could not be established in such hosts. These data suggest that the *entB* gene is not capable of high-frequency translocation.

In other communications (8, 9) we showed that the genetic determinant for staphylococcal enterotoxin B (*entB*) can be either plasmid borne or chromosomal. About one-third of the strains analyzed possessed the plasmid genotype, and all of these were methicillin resistant (Mec^r). The remaining two-thirds of the strains possessed chromosomal determinants of staphylococcal enterotoxin B (SEB) and were either Mec^r and Mec^s.

Genetic analysis showed that the *entB* gene could only be studied in those strains that were Mec^r. In fact, methicillin resistance and SEB cotransduced with high frequency (8-10), although they do not appear to be closely linked genes (9). Furthermore, the mobility of the *entB* gene from either plasmid or chromosomal donors seems dependent upon cotransfer of *mec* and a tetracycline resistance plasmid.

Methicillin resistance has already been suggested to be a translocatable genetic element (11). The strong association exhibited by *mec* and *entB* and the plasmid-chromosomal genetic duality exhibited by the enterotoxin determinant suggest that *entB* may also possess the ability to translocate. We have examined this possibility by employing a recombination-deficient (*recA1*) mutant as a recipient in the transduction of *entB*. This approach is based on observations from several laboratories (5, 6, 11, 12) that staphylococcal plasmids and high-frequency translocation elements (but not chromosomal genes) are readily established in such hosts.

We compared the ability to transfer the SEB phenotype from plasmid and chromosomal *entB* genes into both *rec*$^+$ and *rec* recipients. The donor and recipient strains employed in this study are listed in Table 1.

We employed strains 8325-4(ϕ11)(pI524) and 8325-4(ϕ11)(pI524) *his-7 recA1* as recipients for *mec* transduction because Cohen and Sweeney (2) showed that *mec* was transduced at high frequencies when the recipient strain contained a 20×10^6-dalton (20-megadalton [Mdal]) penicillinase plasmid (pI524) and was lysogenized by the prophage ϕ11. Strain 8325-4(ϕ11)(pI524) *his-7 recA1* was constructed by transducing pI524 from strain 8325-4(pI524) into 8325-4(ϕ11) *his-7 recA1*. The *recA1* genotype was confirmed by the inability to induce ϕ11 (16) and the inability to serve as a recipient for a chromosomal gene (*nov*). The stringency of the *recA1* genotype was evident by the ability to be transduced for plasmid-associated tetracycline resistance (Tc^r) at a frequency of 10^{-6} and the inability to obtain novobiocin-resistant transductants (using ISP2 as the donor; frequency, $<10^{-10}$).

The preparation of phage 29 transducing lysates and transduction has been described elsewhere (8, 9). Tetracycline-resistant (Tc^r) transductants were selected on Trypticase soy agar plates containing 5 μg of tetracycline per ml and were screened for the phenotype by replicating onto agar plates containing 5 μg of methicillin per ml and 5% NaCl. The process was reversed for primary selection of Mec^r transductants. When Mec^r transductants were desired, the phage preparation was treated with ultraviolet light before infection in order to increase the transduction frequency (2). All Mec^r Tc^r clones were repurified on agar containing both drugs. Enterotoxin production by single colonies was determined immunologically by Elek plate analysis (1, 9) and Laurell immunoelectrophoresis (3, 8, 9) of 36-h culture supernatants. Plasmid deoxyribonucleic acid (DNA) analysis was performed as described by Shafer and Iandolo (9).

In Table 2 it can be seen that Mec^r Tc^r clones from DU-4916 were obtained when either Mec^r or Tc^r was initially selected. However, when

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strain COL was the donor of Mec^r and Tc^r, the double transductants were only obtained when the initial selection was for Tc^r. All Mec^r Tc^r clones were screened for SEB production. The results demonstrate that when DU-4916 (which hosts pEntB) served as the donor, toxin synthesis was cotransduced with Mec^r and Tc^r equally well into either the *recA1*$^+$ or *recA1* recipient. With primary selection for Mec^r, 30% of the Tc^r cotransductants were SEB$^+$ in the *recA1* host, whereas 52% of the *recA1*$^+$ cotransductants were SEB$^+$. When Tc^r was used for primary selection with the *recA1* host, 100% of the Mec^r cotransductants were SEB$^+$. The corresponding control showed 30% SEB$^+$ Tc^r Mec^r cotransductants.

When strain COL (chromosomal *entB* genotype) served as the donor for Mec^r and Tc^r, SEB$^+$ transductants were only obtained when the *recA1*$^+$ recipient was employed. To determine whether the Mec^r Tc^r *recA1* transductants obtained from strain COL produced low levels of SEB which were not detected by Elek plate analysis, several clones were propagated in 3% NP broth for 36 h, and the culture supernatants were concentrated 50-fold by pervaporation (3). The concentrated samples were analyzed for SEB by immunoelectrophoresis and were found to be devoid of toxin.

Agarose gel electrophoresis of cleared lysates from donor strains, recipient strains, and transductants was performed to verify the plasmid profiles of each clone. The agarose gel profile of the donor strains (DU-4916 and COL), the recipient strain 8325-4(φ11)(pI524) *his-7 recA1*, and the transductants is presented in Fig. 1. The plasmid DNA profile of the parent strain DU-4916 is presented in lane A, and its SEB$^-$ and SEB$^+$ derivatives of 8325-4(φ11)(pI524) *his-7 recA1 mec tc* are presented in lanes E and F. Both clones were found to contain the resident pI524 plasmid and the transduced Tc^r plasmid. However, the SEB$^+$, but not the SEB$^-$, derivative also contained the 1.15-Mdal pEntB plasmid present in strain DU-4916. Similar gel profiles were obtained when the *recA1*$^+$ transductants from strain DU-4916 were analyzed for the presence of plasmid DNA (data not presented).

The plasmid DNA profile of the transductants using strain COL as the donor of *mec* and *tc* was also analyzed. The 3-Mdal plasmid responsible for tetracycline resistance in strain COL (lane B) was resolved in all transductants resistant to tetracycline (lane D). Furthermore, SEB$^+$

TABLE 1. *Bacterial strains used in this study*

Strain	Relevant genotype[a]	Source
DU-4916	*cd bla tc mec entB*	S. Cohen
COL	*tc mec entB*	B. Wilkinson
8325-4 (pI524)	*cad bla*	R. P. Novick
8325-4 (φ11)	φ11	P. A. Pattee
8325-4 (φ11) (p1524)	φ11 *bla cad*	This laboratory
8325-4 (φ11) *his-7 recA1*	φ11 *recA1*	R. P. Novick
8325-4 (φ11) (pI524) *his-7 recA1*	φ11 *bla cad recA1*	This laboratory
ISP2	8325 *nov*	P. A. Pattee

[a] Abbreviations are as follows: *cd*, cadmium resistance; *bla*, beta-lactamase production; *tc*, tetracycline resistance; *mec*, methicillin resistance; *entB*, enterotoxin B; *nov*, novobiocin resistance; φ11, prophage φ11.

TABLE 2. *Transduction studies using a recombination-deficient mutant*

Donor	Recipient	Selected phenotype[a]	Cotransduction	Mec/Tc	% SEB	Plasmid profile[b]	
						SEB$^+$	SEB$^-$
DU-4916	*recA1*$^+$	Mec^r, 6.5×10^{-7} (650)	Tc^r, 5% (33/650)	3.3×10^{-8}	52 (17/33)	20/3/1.15	20/3
DD-4916	*recA1*	Mec^r, 4.9×10^{-7} (490)	Tc^r, 4.7% (23/490)	2.3×10^{-8}	30.4 (7/23)	20/3/1.15	20/3
DU-4916	*recA1*$^+$	Tc^r, 4.5×10^{-6} (4,500)	Mec^r, 0.75% (34/4,500)	3.4×10^{-8}	30 (10/34)	20/3/1.15	20/3
DU-4916	*recA1*	Tc^r, 1.47×10^{-6} (971)	Mec^r, 0.65% (6/971)	9.0×10^{-9}	100 (6/6)	20/3/1.15	20/3
COL	*recA1*$^+$	Mec^r, 2.6×10^{-7} (260)	Tc^r, 0%		0		20
COL	*recA1*	Mec^r, 6.9×10^{-7} (690)	Tc^r, 0%		0		20
COL	*recA1*$^+$	Tc^r, 1.3×10^{-6} (1,300)	Mec^r, 0.92% (12/1,300)	1.2×10^{-8}	83 (10/12)	20/3	20/3
COL	*recA1*	Tc^r, 2.78×10^{-6} (2,780)	Mec^r, 0.65% (18/2,780)	1.8×10^{-8}	0 (0/100[c])		20/3

[a] Frequencies are expressed as the number of transductants per plaque-forming unit. Parentheses indicate number of clones screened.

[b] Expressed in megadaltons as determined by agarose gel electrophoresis. Numbers are three different molecular weights of the three plasmids under consideration.

[c] Total number of Mec^r Tc^r transductants screened from at least three separate experiments.

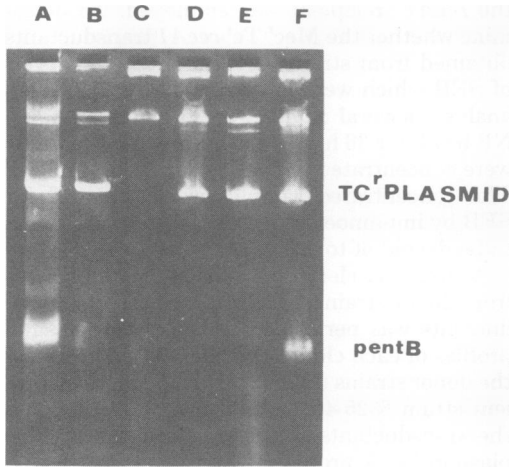

FIG. 1. *Agarose gel electrophoresis of cleared lysate DNA. (A) Staphylococcus aureus DU-4916; (B) S. aureus COL; (C) S. aureus 8325-4(φ11)(pI524) his-7 recA1; (D) 8325-4(φ11)(pI524) his-7 recA1 mec tc; (E) 8325-4(φ11)(pI524) his-7 recA1 mec tc; (F) 8324-4(φ11)(pI524) his-7 recA1 mec tc entB. The transductant presented in (D) was obtained using S. aureus COL as the donor for Mec^r Tc^r; S. aureus strain DU-4916 was the donor for the clones presented in (E) and (F). Details regarding the conditions of electrophoresis are presented in reference 7.*

(*recA1*$^+$) and SEB$^-$ (*recA1*$^+$ and *recA1*) transductants resistant to both methicillin and tetracycline had the same plasmid DNA profile. In other experiments, transductants resistant to tetracycline but not methicillin also harbored the 3-Mdal plasmid. A summary of the genetic and plasmid DNA results obtained is presented in Table 2.

The finding that *entB* from strain DU-4916 was established in both 8325-4(φ11)(pI524) and the *recA1* derivative confirms previous reports (9, 10) that the gene is plasmid borne in this strain. Additionally, the association of *entB* with the 1.15-Mdal plasmid is evident.

We recognize that translocation of chromosomal *entB* was not unequivocally examined because of the inability to select directly for SEB$^+$ transductants. However, these experiments in the *recA1* recipient confirm the lack of plasmid linkage in chromosomal strains and indicate that any potential independent translocation of *entB* must occur at a very low and presently undetectable frequency. That is, if the transduction frequency of 10^{-8} for *entB* noted in this work (from strain COL to the *recA1*$^+$ recipient; Table 2) is multiplied by the probability of translocation of other staphylococcal transposons of about 10^{-4} per donor genome (7), then the combined probability of 10^{-12} is well below the limits of detection of this system. Indeed, this same argument was recently stated by Phillips and Novick (7) to explain the inability to detect translocation of Tn 551 in *recA1* recipients by transduction.

In contrast, the ability to establish chromosomal methicillin resistance in the *recA1* recipient suggests that this gene does translocate at high frequency. Further, this finding underscores the absence of linkage between *mec* and *entB*, which we reported earlier (9), even though such linkage seems evident in the wild type (Table 2).

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