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Increase in Transduction Efficiency of Tn*551* Mediated by the Methicillin Resistance Marker

BRIGITTE BERGER-BÄCHI

Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

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The transduction efficiency of Tn*551*, a staphylococcal transposon coding for erythromycin resistance (*ermB*), was increased by a factor of about five in acceptor strains carrying the gene for methicillin resistance (*mec*), as compared with methicillin-sensitive strains. This enhancement was independent of the generalized transducing phage used and of the chromosomal insertion site of the Tn*551* transposon.

The methicillin resistance gene (*mec*) in staphylococci has been reported to be chromosomally located (3), probably residing on an extra piece of DNA not present in methicillin-sensitive (Mec^s) strains (7). However, based on its genetic behavior, it might be integrated into a transposable element (2). The methicillin resistance shows an unexpected interaction in promoting the transduction efficiency of the Tn*551* transposon. This activity might indicate some recombinational functions encoded or activated by the methicillin resistance gene.

The strains used in this study are described in Table 1. A set of isogenic strains, all derivatives of strain NCTC8325 and varying in just one factor, were constructed to see whether the genetic background of the strain carrying the Tn*551* insertion had any influence on transduction efficiency of the transposon. As mediators for erythromycin resistance, the transducing phages (29 and 80α) were grown on strains BB285, BB291, BB293, and BB296. Strains BB285, BB291, and BB293 had the same Tn*551* insert. In strain BB296, Tn*551* is located at another site. This had been verified by Southern blots (6) with radioactively labeled Tn*551* DNA to *Eco*RI digests of the chromosomal DNA (data not shown). Strain BB291 is a Mec^r derivative of strain BB285; both strains carry the three prophages φ11, φ12, and φ13, whereas strains BB293 and BB296 are phage-free. Three Mec^s strains were used as acceptor strains for the transduction, viz., RN450, a prophage-free strain, FK268, a strain with the three prophages and the penicillinase plasmid pI524, and BB255, which is a plasmid-free derivative of FK268. The two Mec^r acceptor strains, BB262 and BB270, were derivatives of strain BB255.

The transduction frequencies for erythromycin resistance obtained after UV irradiation of the different lysates are shown in Fig. 1. The irradiation time of 10 min corresponded to a reduction of the plaque-forming ability of the transducing lysates by approximately 90% (data not shown). UV irradiation of the transducing lysates increases the frequency of stable transductants for chromosomal genes (1), and a maximum is observed after a 90% reduction of the PFU. This effect was observed also in this model system. There was no decrease of the erythromycin resistance transduction efficiency after 10 min of UV irradiation of the transducing lysate, which reduced the phage viability by 90%. But as a rule, erythromycin-resistant (Em^r) transductants were obtained in higher proportions when the acceptor strain carried a *mec* determinant. The transducing frequencies with phage 29 were somewhat lower than those with phage 80α, but the effect was the same. The increase in transduction efficiency was independent of the genetic background of the donor. Two strains with different *mec* determinants and isogenic except for the region of DNA carried along with the *mec* determinant in the transducing phage showed the same increase in transduction efficiency. Where tested, the Mec^s strains RN450, FK268, and BB255 gave similar transducing efficiencies for erythromycin resistance, with no marked increase after the UV irradiation of the phage. The influence of prophages or of the plasmid content of an acceptor strain could therefore be ruled out. The increase of the transduction efficiencies in Mec^r strains was 3- to 13-fold, with a mean of about 5-fold (Table 2). The low value for strain BB262 with phage 80α grown on strain BB293 was an exception. The transduction of whole plasmids seemed not to be affected by the *mec* determinant. Plasmid pBB2,

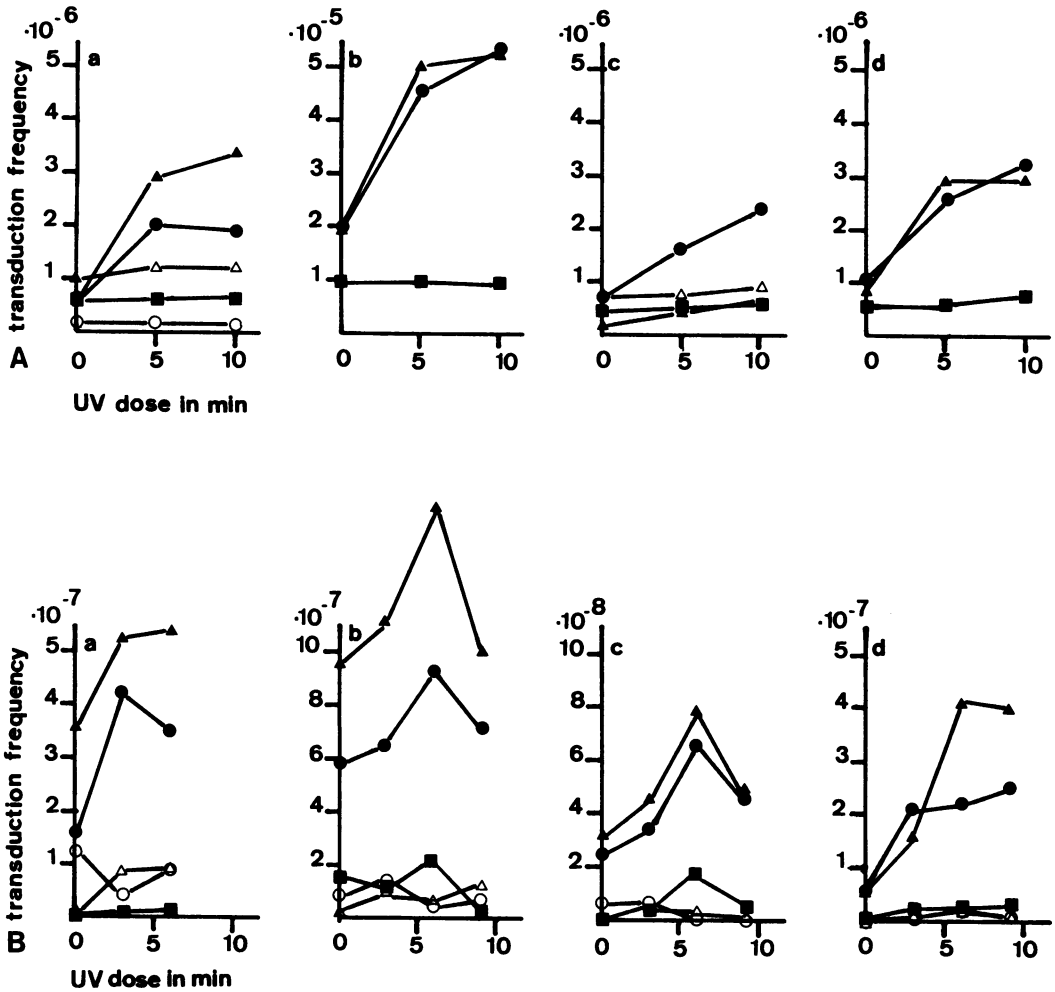

FIG. 1. Transduction frequencies of Tn*551*. The transducing phages were grown and propagated by the agar overlay method. UV irradiation for 10 min reduced the plaque-forming ability of the transducing lysate by 90%. A 0.3-ml portion of an overnight culture of an acceptor strain was exposed to various dilutions of the irradiated phages for 15 min at 37°C, and the transductants were scored on brain heart infusion plates containing 20 μg of erythromycin per ml. The phages used were 80α (A) and 29 (B) grown on strains BB285 (a), BB291 (b), BB293 (c), and BB296 (d). The acceptor strains were the Mec^s strains RN450 (○), FK268 (△), and BB255 (■) and the Mec^r strains BB270 (●) and BB262 (▲).

a 14-kilobase, Tn*551*-carrying plasmid was transduced with the same efficiency into strain BB270 as into strain BB255 (0.9×10^{-4} and 1.3×10^{-4}, respectively). The increased transduction efficiency was restricted to the transposon Tn*551* when chromosomally located. The transduction frequencies of *purA*, a chromosomal marker, into a Mec^r (BB295) or Mec^s (BB359) recipient were identical (3.1×10^{-6} and 3.8×10^{-6}, respectively). The increased efficiency might be due to an increase in recombination functions either provided by or directed by the *mec* determinant. Further studies will be needed to elucidate the various functions of the methicillin resistance determinant.

TABLE 1. Strains used

Strain	Genotype	Origin
RN450	Same as for NCTC 8325-4	R. P. Novick (5)
FK268	Same as for NCTC 8325 (pI524)	S. Cohen (4)
BB255	Same as for NCTC 8325	This laboratory; FK268 cured from pI524 by ethidium bromide
BB262	Same as for NCTC 8325, *mec*	This laboratory, by transduction of strain BB255 with phage 83α-grown on strain E691 *mec* originally isolated from a patient
BB270	Same as for NCTC 8325, *mec*	This laboratory, by transduction of strain BB255 with phage φ11 grown on strain E142 *mec* originally isolated from a patient
BB285	Same as for NCTC 8325, Ω2000 (*chr*::Tn*551*)	This laboratory; Em^r Mec^s derivative of strain BB270, obtained by heat inactivation of a temperature-sensitive plasmid carrying Tn*551* (pRN3208; R. P. Novick) previously introduced into strain BB270; loss of Mec^r not yet further characterized
BB291	Same as for NCTC 8325, Ω2000 (*chr*::Tn*551*) *mec*	This laboratory, by transduction of strain BB270 with phage 80α grown on strain BB285
BB293	Same as for NCTC 8325-4, Ω2000 (*chr*::Tn*551*)	This laboratory, by transduction of strain RN450 with phage 80α grown on strain BB285
BB296	Same as for NCTC 8325-4, Ω2002 (*chr*::Tn*551*)	This laboratory, by heat inactivation of a temperature-sensitive plasmid carrying Tn*551* (pRN3208; R. P. Novick) previously introduced in strain RN450
BB295	Same as for NCTC 8325, *mec purA102 nov-142*	This laboratory, by transduction of strain BB270 with phage 80α grown on strain ISP86 (NCTC 8325 *nov-142 uraA141 hisG15 purA102 pig-131*; P. Pattee)
BB359	Same as for NCTC 8325, *purA102 nov-142*	This laboratory, by transduction of strain BB255 with phage 80α grown on strain BB295
BB303	Same as for NCTC 8325-4(pBB2)	This laboratory, by transformation of strain RN450 with the largest *Eco*RI fragment of φ11de which harbors Tn*551*

TABLE 2. Enhancement of the transduction efficiency of Tn*551* in Mec^r strains as compared with Mec^s strains

Transducing phage	Acceptor strains (Mec^r/Mec^s)	Enhancement valve[a] with the following donor strain for Tn*551*:			
		BB285	BB291	BB293	BB296
80α	BB270/BB255	3.1	5.2	4.4	3.2
80α	BB262/BB255	5.3	5.4	1.0	4.1
29	BB270/BB255	3.6	4.3	3.5	8.3
29	BB262/BB255	4.5	7.1	4.2	13.8

[a] The enhancement value was calculated by dividing the highest transduction frequencies obtained for erythromycin resistance in the Mec^r strains BB270 and BB262 by the frequency obtained in the isogenic Mec^s strain BB255.

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