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Biological Characteristics of a Type I Restriction-Modification System in *Staphylococcus aureus*

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Two restriction-modification systems, S1 and S2, are present in *Staphylococcus aureus* RN450 (S. Iordanescu and M. Surdeanu, J. Gen. Microbiol., **96**:277-281, 1976). System S2 affects phage multiplication after both infection and transfection. Unmodified plasmid and chromosomal DNAs are also not expressed following transduction and transformation into a restrictive host. Restricted phages are, however, capable of conferring phage-mediated competence, although the state of competence does not affect the restriction-modification system. The restricting activity of system S2 is inactivated by heat treatment of the cells. An enzymatic activity that restricts unmodified phage DNA in the presence of ATP, Mg^{2+}, and *S*-adenosylmethionine was recovered from cell-free extracts of a strain RN450 derivative.

Restriction and modification systems have been observed in many taxonomically unrelated bacteria (1, 4, 14). There are two different types of restriction-modification (R-M) enzymes. The restriction and modification enzymes present in *Escherichia coli* B, type I enzymes, are structurally complex and require ATP, Mg^{2+}, and *S*-adenosylmethionine (SAM) for endonucleolytic activity (5, 12, 15, 21). Restriction and modification by the type II enzymes have been observed in numerous bacteria (20). These enzymes are small (less than 100,000 daltons) and require only Mg^{2+} as cofactor. One restriction endonuclease of the latter type, *Sau* I3A, has recently been isolated from *Staphylococcus aureus* 3A (28).

Restriction of lytic phage in *S. aureus* has been extensively studied (13, 19, 22). Patterns of infection of *S. aureus* strains with phages of the International Phage Typing system depend on R-M systems and lysogeny (27).

It has long been known that many untypeable strains of *S. aureus* become typeable after heat treatment (3, 27). Stobberingh et al. (27) demonstrated heat sensitivity of a type II class enzyme of *S. aureus* (28). There are many R-M systems involved in *S. aureus*, and most strains carry several of them (27).

Restriction mutants of *S. aureus* and two R-M systems (S1 and S2) have recently been identified in strain RN1 (10, 27). This communication describes the characteristics of the S2 R-M system in *S. aureus* U224. Phage sensitivity, transfection, and transfer of plasmids and chromosomal markers by transduction and transformation were used to define the restriction system. An endonuclease activity was isolated, which probably is a component of a type I R-M system (S2) also present in strain RN450.

MATERIALS AND METHODS

Bacteria and phages. The *S. aureus* strains are described in Table 1. All phages besides phages ϕ11 and ϕ11*vir* (16, 24) and their propagating strains were obtained from the National Bacteriological Laboratory, Sweden. According to their patterns of phage sensitivity, the strains are divided in four groups (I through IV) (31). Propagation and purification of phages have been described earlier (23, 24).

Media and growth. Media for cultivation of bacteria, phage propagation, and the competence buffer for transformation and transfection have been described (24). RN981 and its derivatives were grown in brain heart infusion broth, and transformants and transductants were selected on brain heart infusion agar.

Preparation of DNA. Bulk DNA (25) and cleared lysates (17) were prepared by published procedures. The covalently closed circular DNA was isolated from cesium chloride-ethidium bromide gradients. After threefold dilution of the samples from the gradients in TE buffer [0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5)-1 mM ethylenediaminetetraacetic acid (EDTA)], ethidium bromide was extracted with water-saturated *n*-butanol. DNA was precipitated with ethanol and collected by centrifugation in a Spinco SW50.1 rotor at 30,000 rpm for 30 min. DNA was dissolved in TE buffer and kept at 4°C.

Transduction. A volume of 1.0 ml of the recipient strain (10^9 colony-forming units/ml) was centrifuged, and the pellet was suspended in the same volume of a transducing lysate of phage ϕ11 containing 2×10^9 to 4×10^9 plaque-forming units/ml. $CaCl_2$ (4 mM) was added, and after 20 min at 37°C the mixture was centrifuged. The pellet was suspended in 1.0 ml of

TABLE 1. *Strains of S. aureus used in this investigation*

Strain	Genotype	Derivation and description	Source and reference
ISP2	8325 *nov*		P. A. Pattee
PS3A		Propagating strain for phage 3A	NBLS[a]
PS83A		Propagating strain for phage 83A	NBLS
PS42D		Propagating strain for phage 42D	NBLS
PS80		Propagating strain for phage 80	NBLS
PS80CR3		Restrictionless mutant of PS80 cured of prophages; sensitive to all group I, II, and III phages	E. E. Stobberingh (27)
RN1	8325	See reference	R. P. Novick (16)
RN450	8325-4	See reference	R. P. Novick (16)
RN981	8325-4 *his7 uvr recA1*	See reference	R. P. Novick (16)
U202	8325-4 (φ11, 83A)	See reference	Our laboratory (26)
U203	8325-4 (φ11) pI258	See reference	Our laboratory (25)
U210	9325-4 (φ11) *mec str tet cad nov*	See reference	Our laboratory (25)
U212	8325-4 (φ11) (83A) *his7 uvr recA1*	See reference	Our laboratory (25)
U215	8325-4 (pI258) *his7 uvr recA1*	From U203 by φ11 transduction	Our laboratory
U218	8325-4 (pI258)	From U203 by φ11 transduction	Our laboratory
U219	8325-4 (φ11, 83A) *his7 uvr*	Spontaneous Rec^+ revertant of U212	Our laboratory
U220	8325-4 (φ11, 83A) *his7 uvr nov*	From U219 by transformation of bulk DNA ISP2	Our laboratory
U221	8325-4 (φ11) *thy*	Spontaneous aminopterin-enriched mutant of RN450 lysogenized with phage φ11	Our laboratory
U222	8324-4 *his7 uvr recA1 str*	Spontaneous Str^r mutant of RN981	Our laboratory
U223	8325-4 *str*	Spontaneous Str^r mutant of RN450	Our laboratory
U224	8325 *nuc*	See reference	Our laboratory (24)

[a] NBLS, Swedish National Bacteriological Laboratory.

Trypticase soy broth medium. Samples (0.1 ml) were spread on selective plates. The screening methods used have been described before (25). The following controls were included: (i) plating of the recipient without phages and (ii) sterility of phage preparations.

Transformation. Competent cells were prepared as described earlier (23). A 0.1-ml volume of bacteria (8×10^8 colony-forming units/ml) in competence buffer was incubated with 1 to 2 μg of covalently closed circular DNA for 10 min at 30°C in the presence of superinfecting phages at a multiplicity of infection of 20. The mixture was centrifuged, and the pellet was suspended in 0.2 ml of Trypticase soy broth and spread on selective plates. When chromosomal genes were transformed, 10 to 20 μg of bulk cellular DNA was added to 0.5 ml of competent cells. The controls included: (i) plating of the recipient without DNA and (ii) sterility of the DNA preparation.

Transfection. The transfection procedure has been described previously (24).

Preparation of crude extract from strain U224. Three liters of Trypticase soy broth medium was inoculated with an overnight culture of strain U224. After incubation for 6 h at 37°C, the cells were centrifuged and washed twice with 0.1 M Tris-hydrochloride (pH 7.5) containing 0.15 M EDTA. The cells (about 7 g) were resuspended in 100 ml of 50 mM Tris-hydrochloride (pH 7.5) containing 2.5 M NaCl and 50 mM EDTA. Lysostaphin was added to a final concentration of 50 μg/ml. After 5 to 10 min at 37°C, the protoplasts were centrifuged and lysed by resuspension in 100 ml of 10 mM Tris-hydrochloride (pH 7.0) containing 0.1 mM EDTA, 0.1 mM β-mercaptoethanol, and 5% glycerol (A buffer). The cells were then disrupted by sonic treatment at 0°C (five 1-min periods with an MSE sonicator) and centrifuged for 10 min at 10,000 rpm in a Sorvall GSA rotor at 4°C. The supernatant fluid was collected, and streptomycin sulphate (0.037 g/ml) was added. The precipitate was removed by centrifugation at 16,000 × g for 10 min at 4°C. $(NH_4)_2SO_4$ was added to 50% saturation, and the precipitate was collected after 30 min by centrifugation at 16,000 × g for 10 min at 4°C. The pellet was dissolved in 5 ml of A buffer, and the crude extract was then dialyzed against two changes of 1,000 ml of A buffer at 4°C.

Fractionation of crude extract on a Bio-Gel A-0.5 m. Solid NaCl was added to the dialyzed ammonium sulphate fraction to a final concentration of 0.5 M, and 0.5 ml of extract was layered on an agarose A-0.5 m column (1.5 by 30 cm) equilibrated with 10 mM Tris-hydrochloride (pH 7.0) containing 0.1 mM EDTA, 0.1 mM β-mercaptoethanol, 5% glycerol, and 0.5 M NaCl.

Assay for restrictive activity. The endonuclease activity was studied by a transfection assay; the assay mixture contained 50 μl of phage φ11*vir* DNA (12.5 μg/ml) in 0.01× SSC (1× SSC = 0.15 M NaCl plus 0.015 M sodium citrate), 50 μl of 100 mM Tris-hydrochloride (pH 7.5) containing 10 mM ATP, 10 mM

$MgCl_2$, and 1 mM SAM, and 50 μl of the enzyme fractions. After 2 h of incubation at 37°C, 100 μl of competent cells in competence buffer were added, and after a further 10 min of incubation the number of transfected cells was determined.

RESULTS

Characterization of the S2 R-M system. Iordanescu and Surdeanu (10) have shown that two R-M systems, S1 and S2, are present in strain RN1. Wyman et al. (32) earlier reported that phages propagated on RN981 were restricted and modified in strain RN450, both derivatives of RN1. RN981 is defective in the S2 system (10).

Table 2 shows that both RN450 and RN981 restrict phages propagated on strains belonging to group I, II, and IV, although restriction is reduced in RN981. After treatment at 56°C, the latter strain lost all restriction ability and reduced restriction was observed in RN450. However, phages propagated on RN981 plated on RN450 with an efficiency of plating of 1 after heat treatment of the latter strain. These results suggest that both systems S1 and S2 are sensitive to heat and that the systems are additive.

Effect of competency of host cell on transfection with modified and unmodified DNA. To determine whether the state of competency has any effect on the restricting activity, we analyzed the restriction system by transfection. DNAs of modified ($ms2^+$) and unmodified ($ms2^-$) phage φ11*vir* were prepared and added to competent cells of strain U212 or U202. The results in Table 3 show that the transfection frequency in a restricting recipient is reduced 100-fold with unmodified DNA compared with modified DNA. Thus induction of competence does not in itself cause loss of the host restricting capacity. Preheated competent cells did not restrict unmodified DNA (data not shown).

Competence enhancement with restricted phages. Superinfection with phage 83A may increase the competence of the recipient (23). We therefore determined whether superinfection with unmodified phages could enhance the competence level in a restricting host. Phage 83A propagated on strain RN981 or RN450 was added to transfection mixtures containing $ms2^+$ modified φ11*vir* DNA and strain U212 or U202. Table 4 shows that restricted phages and nonrestricted phages enhanced competence to the same level.

Transduction and transformation of plasmids into a restrictive strain. The penicillinase plasmid pI258 was transferred to a restrictive host by transduction or transformation to investigate whether restriction provides an important barrier against the spread of plasmids among staphylocci. Transducing φ11 phages and covalently closed circular DNA were prepared from derivatives of RN450 and RN981. Table 5 shows that the plasmid is restricted when transduced or transformed into strain U202. The same result was obtained with the tetracycline plasmid pT169 (data not shown).

Restriction of unmodified chromosomal DNA in transduction and transformation. Bulk DNA was prepared from strains U210 and U220 and added to competent cells of strain U221 without any superinfecting phages. After incubation in competence buffer, Thy^+ recombinants were selected on CHM agar (11), and novobiocin-resistant (Nov^r) cells were selected as described earlier (25). Table 6 shows that no transformants were obtained with unmodified DNA when Thy^+ and Nov^r were used as

TABLE 2. *Restriction of phage propagation in strains RN450 and RN981*

Phage	Group	Efficiency of plating			
		RN450	Heated RN450[a]	RN981	Heated RN981[a]
80 · PS80	I	3×10^{-5}	10^{-1}	10^{-3}	1
3A · PS3A	II	$<10^{-10}$	8×10^{-3}	3×10^{-7}	1
83A · PS83A	III	1	1	1	1
42D · PS42D	IV	$<4 \times 10^{-6}$	10^{-1}	10^{-2}	1
83A · RN981	III	10^{-3}	1	1	1

[a] Heated for 2 min at 56°C.

TABLE 3. *Effect of competence of host cell on transfection with modified and unmodified DNA*

Recipient strain[a]	φ11*vir* DNA[b]	Transfection frequency[c]
U212 $rs2^-$	$ms2^+$	4.3×10^{-3}
	$ms2^-$	3.6×10^{-3}
U202 $rs2^+$	$ms2^+$	2.6×10^{-3}
	$ms2^-$	7.5×10^{-6}

[a] $rs2^-$, No restriction against $ms2^-$ DNA; $rs2^+$, restriction against $ms2^-$ DNA.

[b] Modified DNA ($ms2^+$) was obtained from φ11*vir* · RN450; unmodified DNA ($ms2^-$) was obtained from φ11*vir* · RN981.

[c] Transfection frequency, Number of transfectants per recipient.

TABLE 4. *Competence enhancement by superinfection with unmodified phages*

Recipient strain	Superinfection phage 83A	Transfection frequency
U202 $rs2^+$	None	2.0×10^{-6}
	$ms2^+$	2.0×10^{-3}
	$ms2^-$	1.6×10^{-3}
U212 $rs2^-$	None	10^{-7}
	$ms2^+$	1.0×10^{-3}
	$ms2^-$	3.1×10^{-3}

TABLE 5. *Transduction and transformation of modified and unmodified plasmid*[a]

Recipient strain	Phenotype of recipient	Donor strain[b]	Phenotype of donor	Ero^r/ml[c]	
				Transduction	Transformation
U212	rs2$^-$	U215	ms2$^-$	5×10^{-6}	5×10^{-7}
U212	rs2$^-$	U218	ms2$^+$	2.5×10^{-6}	6×10^{-7}
U202	rs2$^+$	U215	ms2$^-$	$<3 \times 10^{-9}$	$<1.0 \times 10^{-9}$
U202	rs2$^+$	U218	ms2$^+$	2.0×10^{-6}	2.0×10^{-7}

[a] Entire plasmid pI258 was transferred. Transductants and transformants were detected by selection for erythromycin resistance.

[b] These strains were either propagating strains for transducing phage ϕ11 or donor strains for covalently closed circular DNA.

[c] Transduction frequency, Number of transductants per phage added; transformation frequency, number of transformants per recipient cell.

TABLE 6. *Transduction and transformation of chromosomal markers with bulk DNA from modified and unmodified strains*

Recipient strain	Phenotype of recipient	Donor strain[a]	Phenotype of donor	Transduction frequency		Transformation frequency	
				Thy$^+$/ml	Str^r/ml	Thy$^+$/ml	Nov^r/ml
U221	rs2$^+$	U210	ms2$^+$	2.0×10^{-6}		10^{-6}	2×10^{-6}
U221	rs2$^+$	U220	ms2$^-$	$<10^{-9}$		$<8 \times 10^{-8}$	$<8 \times 10^{-8}$
U219	rs2$^-$	U210	ms2$^+$				5×10^{-6}
U219	rs2$^-$	U220	ms2$^-$				4×10^{-6}
RN450	rs2$^+$	U222	ms2$^-$		$<10^{-9}$		
RN450	rs2$^+$	U223	ms2$^+$		5×10^{-7}		
PS80CR3	rs2$^-$	U222	ms2$^-$		4×10^{-7}		

[a] These strains were either propagating strains for transducing phage ϕ11 or donor strains for bulk cellular DNA.

markers. The biological activity of unmodified DNA was verified by transfer of Nov^r into strain U219.

ϕ11 phages grown on strain U220 were also unable to transduce the Thy$^+$ marker into rs2$^+$ hosts, showing that this gene(s) is unable to escape restriction both in transformation and transduction. The *str* gene(s) was restricted in the same way. In control experiments, these phages transduced Str^r into the restrictionless mutant PS80CR3.

In vitro restriction with fractionated cell extracts. Cell extracts from strain RN981 and U224 were fractionated as described in Materials and Methods and chromatographed on Bio-Gel A-0.5 m columns. Figure 1A and B show the elution profile of extracts from U224 and RN981, respectively. After digestion with individual fractions, the remaining infectivity of ms2$^+$ and ms2$^-$ ϕ11*vir* DNA was determined by transfection to an rs2$^-$ host. Figure 1A shows that ms2$^-$ DNA is more sensitive than ms2$^+$ DNA for eluate in fractions 18 and 19. The corresponding eluate from RN981 lacking system S2 does not show this difference, suggesting that part of the activity in U224 may represent the S2 restriction enzyme. A second nuclease, which can degrade modified DNA, is present in both extracts and elutes with a peak in fractions 20 to 23 of the chromatogram.

Restricting activity is stimulated by SAM. The requirements for in vitro restriction were determined by using fraction 18 of the column eluate (Fig. 1A). The results (Table 7) show that the restriction endonuclease activity requires Mg^{2+} and ATP and may be stimulated by SAM. In the absence of SAM, a contaminating nuclease degrades about 90% of the DNA when ATP and Mg^{2+} are present. This enzyme is an exonuclease, since transformation by covalently closed circular DNA of the plasmid pT169 was not sensitive (data not shown).

DISCUSSION

Strain RN450 and its derivatives have two R-M systems, S1 and S2 (10). Both systems restrict phages of groups I, II, and IV. In addition, system S2 restricts phages propagated on RN981. According to Iordanescu and Surdeanu (10), the two systems are not additive in their restricting capacity, in contrast to restriction systems in other species where two R-M systems are present (2, 6, 18). We found that strain RN981, which lacks system S2, does not restrict foreign DNA to the same level as strain RN450. In addition, we were uable to heat inactivate the activity in the latter strain completely. It is unclear whether this is due to alterations in system S1 in mutant RN981 or an additive effect between the two systems. Phage sensitivity has

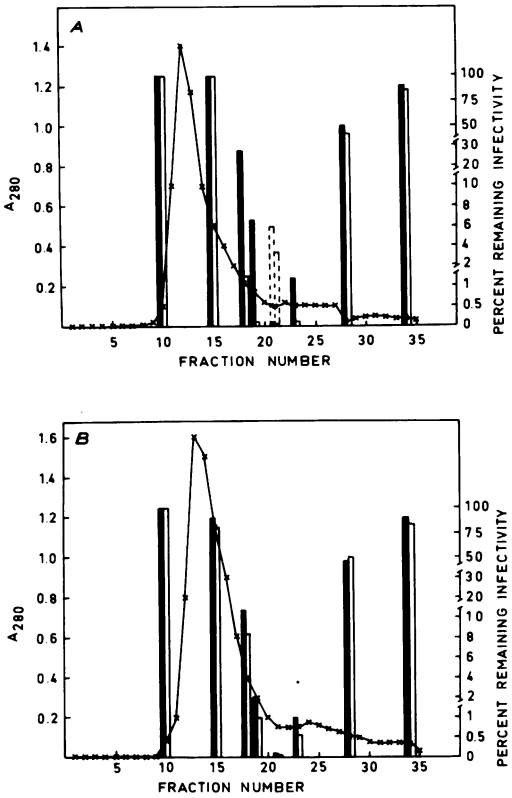

FIG. 1 *A, B. Partial purification of cell extract of U224 (A) and RN981 (B) on Bio-Gel A-0.5 m (1.5 by 30 cm). A volume of 0.5 ml of extract in A buffer containing 0.5 M NaCl was layered on the column and eluted with the same buffer at a flow rate of 1.4 ml/h. A volume of 50 μl of each 1.4-ml fraction was then assayed for restricting activity by transfection with ms2⁻ and ms2⁺ φ11vir DNA to a nonrestricted host. (×) Absorbance at 280 nm (A_{280}). filled bars, ms2⁺, and unfilled bars, ms2⁻ φ11vir DNA, expressed as percentage of remaining infectivity. Bars with broken lines denote assays where only 10 μl of fractionated material was used.*

TABLE 7. *Requirements for in vitro restriction*

Reaction mixture[a]	% Remaining infectivity of ms2⁺ φ11*vir* DNA	% Remaining infectivity of ms2⁻ φ11*vir* DNA
Buffer (100 mM Tris-hydrochloride, pH 7.4)	90	89
+ ATP	84	89
+ ATP + SAM	89	92
+ ATP + Mg^{2+}	11	5
+ Mg^{2+}	90	85
+ Mg^{2+} + SAM	88	83
+ Mg^{2+} + SAM + ATP	12	0.3

[a] Final concentrations, 3.3 mM ATP, 3.3 mM Mg^{2+}, and 0.33 mM SAM.

so far been the only tool to study restriction in *S. aureus*. As shown here and previously for other R-M systems in other species (7, 8), system S2 also prevents expression of unmodified DNA in transfection assays, suggesting that the state of competency does not affect the restricting properties in derivatives of strain RN450.

Unmodified phage 83A induces optimal competence by superinfection in a restricting host (Table 4). Unmodified phage λ in *E. coli* complements early *sus* mutants in a restrictive cell (29). We have proposed that the responsible gene(s) for competence induction is an early function of the phage genome (26). This gene(s) may therefore be transcribed and translated before the unmodified DNA becomes degraded.

The sensitivity of unmodified plasmid DNA and chromosomal DNA to restriction was studied by transduction and transformation. The penicillinase plasmid pI258 and the tetracycline plasmid pT169 were both restricted after transfer into a restricting host, suggesting that the restriction enzyme is an endonuclease capable of degrading covalently closed circular DNA. Thus restriction enzyme(s) may provide an effective barrier against the spread of resistant plasmids between staphylococci in vivo.

Unmodified chromosomal DNA is also restricted in transduction and transformation in *S. aureus* (Table 6). In *Bacillus subtilis*, on the other hand, transformation of chromosomal DNA is not subject to restriction (30). This difference in sensitivity may be a function of whether DNA enters the cell as a double- or single-stranded DNA.

Crude cell extracts of strain U224 contain a nuclease activity which has specificity for unmodified DNA; i.e., it is more active on unmodified (ms2⁻) DNA than on modified (ms2⁺) DNA (Fig. 1A and Table 7). This enzyme is absent in strain RN981 (Fig. 1B), which lacks the S2 R-M system. The restricting activity requires ATP and Mg^{2+} and may be stimulated by SAM; therefore this nuclease may be a representative of the type I class enzymes.

Restrictionless mutants of *S. aureus* have been isolated (10, 27), and these mutants show a new pattern of phage sensitivity. Nearly all restriction-deficient mutants isolated by Stobberingh et al. (27) were still able to modify DNA, and Sussenbach et al. (28) have recently isolated a type II class enzyme of *S. aureus* strain 3A. From these results they conclude that R-M systems in *S. aureus* are not of the type I class. On the contrary, Iordanescu and Surdeanu (10), working with a derivative of strain RN450, obtained a high proportion of m^- mutants among isolated r^- mutants. In accordance with the three-gene model (9), they suggested that both

R-M systems in strain RN450 are type I. Our results suggest that the S2 restriction nuclease is a type I class enzyme.

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LITERATURE CITED

1. **Arber, W., and S. Linn.** 1969. DNA modification and restriction. Annu. Rev. Biochem. **38**:467-500.
2. **Arber, W., and D. Wauters-Willems.** 1970. Host specificity of DNA produced by *Escherichia coli*. XII. The two restriction and modification systems of strain 15T⁻. Mol. Gen. Genet. **108**:203-217.
3. **Asheshov, E. H., and M. P. Jevons.** 1963. The effect of heat on the ability of a host strain to support the growth of a staphylococcus phage. J. Gen. Microbiol. **31**:97-107.
4. **Boyer, H. W.** 1971. DNA restriction and modification mechanisms in bacteria. Annu. Rev. Microbiol. **25**:153-176.
5. **Boyer, H. W.** 1974. Restriction and modification of DNA: enzymes and substrates. Fed. Proc. **5**:1125-1127.
6. **Colson, C., and A. van Pel.** 1974. DNA restriction and modification systems in Salmonella. I. SA and SB, two *Salmonella typhimurium* systems determined by genes with a chromosomal location comparable to that of the *Escherichia coli hsd* genes. Mol. Gen. Genet. **129**:325-337.
7. **Dussoix, D., and W. Arber.** 1965. Host specificity of DNA produced by *Escherichia coli*. IV. Host specificity of infectious DNA from bacteriophage lambda. J. Mol. Biol. **11**:238-246.
8. **Gromkova, R., J. Bendler, and S. Goodgal.** 1973. Restriction and modification of bacteriophage S2 in *Haemophilus influenzae*. J. Bacteriol. **114**:1151-1157.
9. **Hubacek, J., and S. W. Glover.** 1970. Complementation analysis of temperature-sensitive host specificity mutations in *Escherichia coli*. J. Mol. Biol. **50**:111-127.
10. **Iordanescu, S., and M. Surdeanu.** 1976. Two restriction and modification systems in *Staphylococcus aureus* NCTC 8325. J. Gen. Microbiol. **96**:277-281.
11. **Lindberg, M., J.-E. Sjöström, and T. Johansson.** 1972. Transformation of chromosomal and plasmid characters in *Staphylococcus aureus*. J. Bacteriol. **109**:844-847.
12. **Linn, S., J. A. Lautenberger, B. Eskin, and D. Lackey.** 1974. Host-controlled restriction and modification enzymes of *Escherichia coli* B. Fed. Proc. **5**:1128-1134.
13. **Ma, P., and R. J. Mandle.** 1961. Phage typing nontypable strains of *Staphylococcus aureus*. Bacteriol. Proc. **111**:10-17.
14. **Meselson, M., R. Yaun, and J. Heywood.** 1972. Restriction and modification of DNA. Annu. Rev. Biochem. **41**:447-466.
15. **Nathans, D., and H. O. Smith.** 1975. Restriction endonucleases in the analysis and restructuring of DNA molecules. Annu. Rev. Biochem. **44**:273-293.
16. **Novick, R. P.** 1967. Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. Virology **33**:155-166.
17. **Novick, R. P., and D. Bouanchaud.** 1971. Extra chromosomal nature of drug resistance in *Staphylococcus aureus*. Ann. N.Y. Acad. Sci. **182**:279-294.
18. **Piekarowicz, A., and S. W. Glover.** 1972. Host specificity of DNA in *Haemophilus influenzae*: the two restriction and modification systems in strain Ra. Mol. Gen. Genet. **116**:11-25.
19. **Ralston, D. J., and B. S. Baer.** 1964. A new property of phage group II *Staphylococcus aureus* strains: host restriction of phage K14. J. Gen. Microbiol. **36**:1-16.
20. **Roberts, R. J.** 1976. Restriction endonucleases. CRC Crit. Rev. Biochem. **4**:123-164.
21. **Roulland-Dussoix, D., and H. W. Boyer.** 1969. The *Escherichia coli* B restriction endonuclease. Biochim. Biophys. Acta **195**:219-229.
22. **Rountree, P. M.** 1956. Variations in a related series of staphylococcal bacteriophages. J. Gen. Microbiol. **15**:266-279.
23. **Sjöström, J.-E., M. Lindberg, and L. Philipson.** 1972. Transfection of *Staphylococcus aureus* with bacteriophage deoxyribonucleic acid. J. Bacteriol. **109**:285-291.
24. **Sjöström, J.-E., M. Lindberg, and L. Philipson.** 1973. Competence for transfection in *Staphylococcus aureus*. J. Bacteriol. **113**:576-585.
25. **Sjöström, J.-E., S. Löfdahl, and L. Philipson.** 1975. Transformation reveals a chromosomal locus of the gene(s) for methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **123**:905-915.
26. **Sjöström, J.-E., and L. Philipson.** 1974. Role of the ϕ11 phage genome in competence of *Staphylococcus aureus*. J. Bacteriol. **119**:19-32.
27. **Stobberingh, E. E. and K. C. Winkler.** 1977. Restriction-deficient mutants of *Staphylococcus aureus*. J. Gen. Microbiol. **99**:359-367.
28. **Sussenbach, J. S., C. H. Monfoort, R. Schiphof, and E. E. Stobberingh.** 1976. A restriction endonuclease from *Staphylococcus aureus*. Nucleic Acid Res. **11**:3193-3202.
29. **Terzi, M.** 1968. Physiology of bacteriophage λ in the restrictive host. J. Mol. Biol. **34**:165-173.
30. **Trautner, T. A., B. Pawlek, and S. Bron.** 1974. Restriction and modification in *Bacillus subtilus*. Biological aspects. Mol. Gen. Genet. **131**:181-191.
31. **Williams, R. E. O., and J. E. Rippon.** 1952. Bacteriophage typing of *Staphylococcus aureus*. J. Hyg. **50**:320-353.
32. **Wyman, L., R. V. Goering, and R. P. Novick.** 1973. Genetic control of chromosomal and plasmid recombination in *Staphylococcus aureus*. Genetics **76**:681-702.