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Regulation of exoprotein expression in *Staphylococcus aureus* by a locus (*sar*) distinct from *agr*

(insertional mutagenesis/global regulator/chromosomal locus/pLTV1)

AMBROSE L. CHEUNG*†, J. MATTHEW KOOMEY*, CHARLES A. BUTLER*, STEVEN J. PROJAN‡, AND VINCENT A. FISCHETTI*

*Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, 1230 York Avenue, New York, NY 10021; and ‡The Public Health Research Institute, 455 First Avenue, New York, NY 10016

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ABSTRACT **A single insertion of transposon Tn*917*LTV1 into the chromosome of a *Staphylococcus aureus* clinical isolate, strain DB, resulted in a pleiotropic effect on the expression of a number of extracellular and cell-wall-associated proteins. Detailed comparison of phenotypes associated with the mutant, 11D2, and the parent, DB, indicated that the chromosomal locus inactivated as a result of transposon mutagenesis differs from the *S. aureus* accessory gene regulator locus (*agr*). In particular, the expression of α-hemolysin, which is not detectable in Agr⁻ mutants, was enhanced in mutant 11D2, while it remained at a low level in strain DB. Likewise, protease activity was significantly enhanced in 11D2 compared with DB. In addition, most of the cell-bound proteins were expressed at lower levels in the mutant than the parent strain. This pattern is contrary to that found in switching from Agr⁺ to Agr⁻ phenotypes. Southern blot hybridization with an *agr* probe indicated that the inactivated chromosomal locus is distinct from *agr*. Transduction experiments demonstrated that the phenotypes associated with mutant 11D2 could be transferred to the parental strain DB as well as to RN450, an *S. aureus* strain with a genetic background similar to strain 8325-4. This locus on the *S. aureus* chromosome, possibly regulatory in nature, has been designated *sar* for staphylococcal accessory regulator.**

Staphylococcus aureus synthesizes a number of extracellular proteins that have been postulated to play a role in bacterial pathogenesis (1). During the postexponential phase of growth, most of these extracellular proteins are rapidly synthesized and are then secreted (1). At the same time, the production of proteins essential for growth is down-regulated. Considerable evidence has now accumulated to suggest that exoprotein genes in prokaryotes may be coordinately regulated (2). This is particularly true in Gram-negative bacteria such as *Vibrio cholerae, Bordetella pertussis, Yersinia, Salmonella*, and *Shigella* species (2). Likewise, evidence for coordinate regulation of exoprotein synthesis has been described in Gram-positive bacteria such as *Bacillus subtilis* (3) and *S. aureus* (4).

Mutations affecting the production of extracellular proteins in *S. aureus* are often pleiotropic (1). A regulatory locus controlling the production of several exoproteins in *S. aureus* has been identified (4) and this locus, alternatively called *agr* for accessory gene regulator (4) and *exp* for extracellular protein (5), acts at the transcriptional level in regulating α-toxin, toxic shock syndrome toxin-1, and other extracellular proteins, many of which are involved in pathogenesis (6). Most of the exoproteins regulated by *agr* are either not synthesized or synthesized at a reduced rate in Agr⁻ mutants, while synthesis of surface proteins is up-regulated. Because of this complexity in the regulation of extracellular and cell wall proteins in *S. aureus*, it has been speculated that other loci may be involved in the regulatory control of exoproteins (6). Searching for a fibrinogen-binding-negative *S. aureus* mutant by using Tn*917*LTV1 mutagenesis, we discovered another locus involved in the global regulation of exoproteins. Phenotypic and molecular analyses suggest that this locus is distinct from *agr*. This unique component, possibly regulatory in nature, has been designated *sar* for staphylococcal accessory regulator.

MATERIALS AND METHODS

Bacteria, Plasmids, and Phage. The bacterial strains and plasmids used in this study are listed in Table 1. Transducing phage φ71 for parent strain DB was kindly provided by P. Pattee (Iowa State University, Ames). Phage φ11 was used as a transducing phage for strain RN4220.

Media and Antibiotics. Unless otherwise indicated, the following media were used: brain heart infusion (BHI) (Difco) for the growth of *S. aureus* and Luria–Bertani broth (LB) for *E. coli*. Antibiotics were used at the following concentrations: carbenicillin at 50 μg/ml for *E. coli* and tetracycline at 5 μg/ml and erythromycin at 10 μg/ml for *S. aureus*.

Plasmid Purification. Plasmid pLTV1 (11), a Tn*917*LTV1 delivery vehicle, was used to transform *E. coli* strain HB101 by standard techniques (ref. 10, pp. 1.74–1.84). The plasmid pLTV1 was purified from *E. coli* strain HB101 using a standard alkaline-SDS miniprep protocol (ref. 10, pp. 1.25–1.28). The purity of the plasmid was confirmed by restriction digest analysis (11). After successful transformation of pLTV1 into an *S. aureus* strain (see below), plasmid DNA was purified from transformed staphylococci that had been lysed with lysostaphin as described (9).

Transformation of *S. aureus*. Protoplast transformation of *S. aureus* was performed as described (9, 12). Transformants were selected at 32°C on DM3 agar (9) containing tetracycline.

Transposon Mutagenesis. The temperature-sensitive plasmid pLTV1 that had been introduced into DB by transformation was used to deliver the transposon Tn*917*LTV1 into the staphylococcal chromosome by shifting the growing temperature from 32°C to 42°C as described (11). To enrich for the selection of fibrinogen-binding-negative and possibly other related mutants, sterile human fibrinogen (75 μg/ml—*S. aureus* clumps at this concentration) was mixed with 5 ml of bacterial suspension (from an overnight 25-ml culture) for 15 min at room temperature. The supernatant containing nonclumping bacteria was plated on BHI agar with erythromycin at 42°C. Individual colonies were then picked and further tested for clumping with fibrinogen in microtiter

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†To whom reprint requests should be addressed.

Table 1. Bacterial strains and plasmids

Strain	Refs.	Comments
	Bacteria	
S. aureus		
DB	7	A wild-type blood isolate
RN4220	8	A mutant of strain 8325-4 (9) that accepts foreign DNA
ISP226		Propagating strain for ϕ71
6A8	This study	A tetracycline-sensitive transformant derived from DB with a Tn*917*LTV1 insert
11D2	This study	A mutant derived from DB with a *sar*::Tn*917*LTV1 mutation
A4	This study	A DB transductant with a *sar*::Tn*917*LTV1 mutation
RN450	8, 9	Propagating strain for ϕ11 with a genetic background similar to strain 8325-4
Wood 46	8	An α-hemolysin-producing strain
Staphylococcus epidermidis 6537		A strain isolated from an infected catheter
Escherichia coli HB101	10	A highly transformable strain used for plasmid replication
	Plasmids	
pLTV1	11	A Tn*917* derivative with a polylinker site and selectable markers for *E. coli* and *S. aureus*
pRN6672	4, 6	pSK265::*agr*-A21 containing a 3.8-kilobase (kb) *Pvu* II *agr* fragment

wells. Strain DB and an *S. epidermidis* isolate were used as positive and negative controls, respectively. The bacteria in wells that did not clump with fibrinogen were tested for tetracycline sensitivity to ensure loss of plasmid and further phenotypically analyzed.

Transduction. To transfer the genetic trait of the mutant 11D2 to parent DB, ϕ71 was first used to produce a phage lysate of the mutant as described (9). The phage lysate was titered and used to infect strain DB at a low multiplicity of infection (phage-to-recipient ratio = 1:10) (9, 13). Transductants were selected on 0.3GL agar (13) with erythromycin. Using a ϕ71 lysate of 11D2, we also transduced the gene of interest in mutant 11D2 to RN4220. A ϕ11 lysate of the RN4220 transductant was then used to infect RN450, an *S. aureus* strain with a genetic background derived from strain 8325-4 (9), to obtain transductants.

Phenotypic Characterizations of Parent, Mutant, and Transductants. Preliminary studies revealed that the growth rates of mutant 11D2 and parent DB in the absence of antibiotics were comparable. For phenotypic characterization, the measurements included hemolysin production (α, β, and γ) on plain and cross-streaked sheep and rabbit erythrocyte agar (4), serine protease assayed by the fibrin agar method (Boehringer Mannheim), coagulase production assayed with 2-fold dilutions of rabbit plasma, lipase production assayed on egg yolk agar and 1% Tween 20 agar (14), DNase production assayed on DNase agar, β-lactamase production assayed by using a chromogenic substrate (nitrocefin) (15), fibronectin-binding protein content as assayed by ^{125}I-labeled fibronectin (^{125}I-fibronectin) binding (16), and the fibrinogen-binding capacity assayed by ^{125}I-labeled fibrinogen (^{125}I-fibrinogen) binding (17).

The extracellular proteins of strain DB, tetracycline-sensitive transformant 6A8, mutant 11D2, and transductant A4 were concentrated 100-fold to obtain their SDS/PAGE protein profiles and relative protein A contents as described (4).

α-Hemolysin in the extracellular fluid was also assayed with 0.5% defibrinated rabbit blood by using an adaptation of the tube method as described by Bernheimer and Schwartz (18). Hemolytic activities were expressed as the reciprocal of the dilution yielding 50% lysis, using *S. aureus* strain Wood 46 as a standard.

A capture ELISA was used to quantitate extracellular protein A. Human IgG (Sigma) at 1 μg/ml was used to coat microtiter wells at 37°C overnight. Serial 4-fold dilutions of concentrated extracellular proteins and Cowan protein A standards (Calbiochem) were added. Protein A bound was assayed by affinity-purified chicken anti-protein A antibody (Accurate Chemicals, Westbury, NY) followed by alkaline phosphatase conjugated to the F(ab')$_2$ fragment of rabbit anti-chicken IgG (Jackson ImmunoResearch) and *p*-nitrophenyl phosphate as a developing substrate.

Cell-wall-associated proteins were extracted with lysostaphin from *S. aureus* cells in a hypertonic medium (30% raffinose) as previously described (7).

SDS/PAGE and Immunoblot Analysis of Extracellular and Cell-Wall-Associated Proteins. Proteins were separated on SDS/9% polyacrylamide slab gels (19). After electrophoresis, proteins were either silver stained (Pierce) or transferred onto nitrocellulose (20) to assay for cell-wall-associated protein A as described (7).

Southern Blot Hybridization. Staphylococcal chromosomal DNA was extracted from lysostaphin-lysed cells as previously described (9, 10). Southern blot hybridization was performed (10) with random-primed or nick-translated samples of gel-purified DNA fragment as probes (10). After prehybridization, DNA on the membrane (Hybond-N$^+$; Amersham) was allowed to hybridize with a ^{32}P-labeled DNA probe at 65°C overnight, washed twice with 2× SSPE with 0.1% SDS at room temperature for 10 min each (9) and once with 1× SSPE (0.15 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) with 0.1% SDS at 65°C for 15 min, and finally autoradiographed.

RESULTS

Transformation of pLTV1 into Wild-Type Blood Isolate Strain DB. Transformation of pLTV1 directly into protoplasts of wild-type DB was unsuccessful in initial studies. To overcome possible restriction barriers and problems related to plasmid replication in the wild-type *S. aureus* isolate, pLTV1 was propagated in HB101 and transformed first into RN4220, an *S. aureus* mutant that is defective in one or more restriction systems (8). Using this approach, we selected seven transformants of RN4220 on DM3 agar containing tetracycline at 32°C. The plasmid pLTV1 was subsequently purified from RN4220. The RN4220-modified pLTV1 was utilized to transform wild-type DB, resulting in two transformants selected on DM3 agar containing tetracycline. The presence of pLTV1 in DB was confirmed by restriction enzyme analysis of plasmid purified from transformed DB (Figs. 1 and 2).

Transposon Mutagenesis. The unique feature of pLTV1 is that it contains a thermosensitive replicon [pE194Ts or pRN5101 (8)] that replicates at 32°C but segregates at 42°C. Growing the transformed bacteria first at 32°C and then at 42°C and selecting erythromycin-resistant colonies yields colonies with Tn*917*LTV1 transposed to the host chromosome. When this approach was used, the transposition frequency was found to be high (5×10^{-4}, or 1 tetracycline-sensitive transformant out of every 2000 colony-forming units plated). With a ^{32}P-labeled *Pst* I fragment of pLTV1 as a probe, transposition of Tn*917*LTV1 was confirmed by a

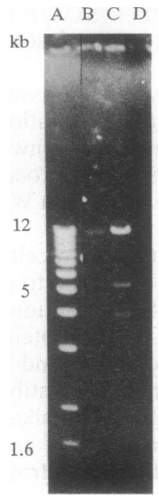

FIG. 1. Agarose electrophoresis of *Eco*RI digests of plasmid pLTV1 purified from transformed parent DB. Lanes: A, DNA marker; B and C, *Eco*RI digest of pLTV1 purified from two DB transformants; D, DB control.

Southern blot of *Eco*RI-digested chromosomal DNA prepared from DB-derived tetracycline-sensitive transformants (Fig. 3*A*). Further analysis indicated that greater than 90% of the insertions of Tn*917*LTV1 were single and appeared to be in different sites (Fig. 3*A*).

For the detection of mutants deficient in fibrinogen-binding capacity and exoprotein production, DB colonies with Tn*917*LTV1 transposed to the host chromosome were first enriched for a fibrinogen-binding-negative phenotype. Individual colonies from the supernatant containing nonclumped bacteria in the presence of fibrinogen were grown in BHI with erythromycin and tested for fibrinogen-binding capacity. Among 17,000 colonies individually screened, 1 tetracycline-sensitive fibrinogen-binding-negative mutant, 11D2, was found. Further phenotypic analysis of this mutant indicated that the expression of a variety of exoproteins was also affected (detailed below). A Southern blot of restriction digests (with *Nco* I and *Eco*RI) of 11D2 chromosomal DNA probed with a 1.5-kb *Hin*dIII fragment internal to the transposon indicated that the mutant contained a single insert (Fig. 3*B*). This reasoning was substantiated by the presence of two hybridizing fragments with the *Nco* I digest. The smaller *Nco* I-hybridizing fragment is internal to the transposon and will remain the same size irrespective of the location of the transposon, whereas the larger fragment is a junction fragment that is composed partly of flanking chromosomal sequence.

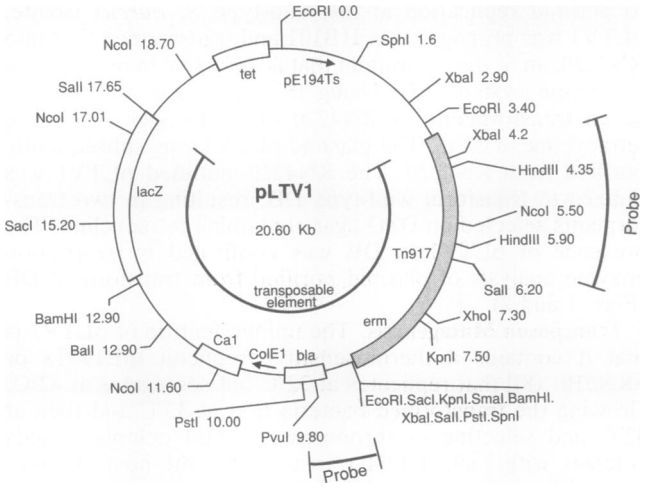

FIG. 2. Restriction map of pLTV1, adapted from Camilli *et al.* (11). The ends of the transposable element as well as the probes used in hybridization studies are marked. Numbers are kb from the *Eco*RI at the top.

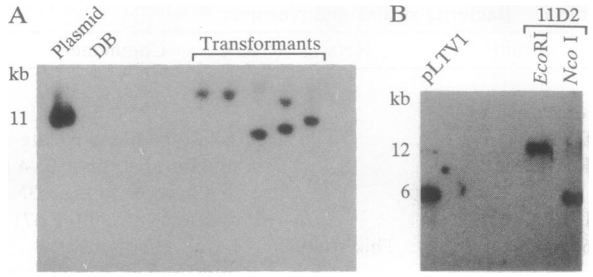

FIG. 3. Southern blots of chromosomal DNA. (*A*) Parent DB and some of the DB-derived tetracycline-sensitive transformants digested with *Eco*RI and probed with a ^{32}P-labeled 1-kb *Pst* I fragment of pLTV1. The two bands in one of the transformants (out of 20 analyzed) indicate a double insertion. (*B*) Mutant 11D2 digested with *Eco*RI and *Nco* I and probed with a ^{32}P-labeled 1.5-kb *Hin*dIII fragment internal to the transposon. The 6-kb *Nco* I-hybridizing fragment is internal to the transposon and the probe (Fig. 2). The plasmid pLTV1 control was digested with *Eco*RI in both of these blots.

Transduction. Transduction was used to determine if the phenotype associated with Tn*917*LTV1 insertion into the mutant chromosome could be transferred back to strain DB. For this procedure, a ϕ71 lysate of 11D2 was used to infect DB at a low multiplicity of infection. Over 100 transductants selected on CYGP agar (9) with erythromycin were obtained. With a 1.5-kb *Hin*dIII fragment of Tn*917*LTV1 as a probe, a Southern blot of chromosomal DNA digested with either *Eco*RI or *Nco* I from 11 of the transductants demonstrated that a single *Eco*RI fragment and two *Nco* I fragments from all transductants hybridized to the probe (data not shown). These hybridizing fragments corresponded to the fragment of 11D2 (see Fig. 3*B*), thus showing that the location of Tn*917*LTV1 insert in the transductants is identical to that of the mutant 11D2.

Phenotypic Characterization of Parent, Mutant 11D2, and Transductant A4. The effect of insertional inactivation by Tn*917*LTV1 on exoprotein expression in 11D2 and one of the transductants, A4, was studied. As shown in Fig. 4, strain 6A8, which has a Tn*917*LTV1 insert in an unrelated chromosomal locus as determined by Southern blot and detailed phenotypic analyses (data not shown), and the parental strain DB exhibited similar extracellular protein profiles after silver stain. However, mutant 11D2 and transductant A4 revealed comparable profiles that are distinct from strain DB. These data indicate that the phenotype associated with a single Tn*917*LTV1 insertion into 11D2 chromosome can be transferred.

The expression of individual exoproteins in mutant 11D2, transductant A4, and parent DB is presented in Table 2. Compared with strain DB, δ-hemolysin was down-regulated,

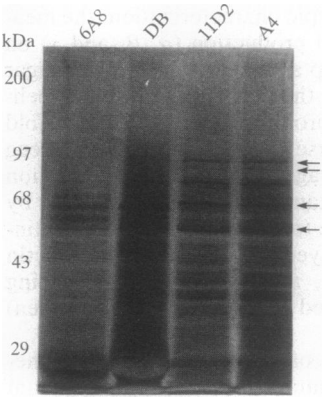

FIG. 4. Silver-stained gel of extracellular proteins (15 μl each of the concentrated proteins) from parent DB, mutant 11D2, transductant A4, and the tetracycline-sensitive transformant control 6A8. Arrows indicate some of the bands either enhanced or found only in mutant 11D2.

Table 2. Phenotypic characterization of the *sar* locus in comparison with known Agr$^+$ and Agr$^-$ phenotypes

Group	Phenotype	Sar$^+$ (DB)	Sar$^-$ (11D2)	Sar$^-$ (A4)	Agr$^+$ (RN6390)	Agr$^-$ (RN6112)
I	δ-Hemolysin	+	−	−	++	−
	Serine protease	+	++	++	++	−
	α-Hemolysin*	174 ± 4	386 ± 62	286 ± 8	2928 ± 85	5 ± 1.3
II	Lipase	+	++	++	+	±
	DNase	++	++	++	++	+
	β-Hemolysin†	−	−	−	++	+
III	Coagulase titer‡	1:16	1:4	1:4	+	++
	Fibrinogen-binding protein§	6833 ± 922¶	1570 ± 217	1867 ± 128	+	+
	Fibronectin-binding protein¶	18715 ± 715	5772 ± 1072	6554 ± 1863	+	++
	Protein A‖					
	Extracellular	0.31	0.03	0.04	+	++
	Cell wall	++	++	++	+	++
IV	β-Lactamase	+	+	+	+	+

The phenotypes for parent DB, mutant 11D2, and transductant A4 were characterized as described in the text. The phenotypes for an Agr$^+$ strain and an Agr$^-$ mutant (ref. 6 and S.J.P., unpublished observations) are also listed for comparison. Strain RN6112 is the original Agr$^-$ mutant with a Tn*551* insertion (6). The expression groups were categorized as described by Kornblum *et al.* (6). Quantitative results are given as mean ± SEM (n = 3).

*Given in hemolytic units/mg of extracellular protein.

†The effect of the *sar* locus on β-hemolysin expression is not testable with these strains. However, strain RN450, a known β-hemolysin producer, fails to express β-hemolysin when transduced with the *sar*::Tn*917*LTV1 mutation.

‡These represent the highest of 2-fold dilutions of rabbit plasma at which coagulation occurs in two independent determinations.

§Data presented as cpm of ^{125}I-fibrinogen bound to 10^9 colony-forming units. The presence of unlabeled fibrinogen (25 μg) in the mixture caused only a 20% reduction in ^{125}I-fibrinogen binding to 11D2 and A4, thus suggesting a relative lack of fibrinogen receptor in the mutant.

¶Values given in cpm of ^{125}I-fibronectin bound to 10^9 colony-forming units. The addition of unlabeled fibronectin (25 μg) resulted in an ≈50% reduction in ^{125}I-fibronectin binding to 11D2 and transductant A4.

‖Quantitative values are given in μg/ml calculated from Cowan I protein A standards. This assay has a sensitivity in the nanogram range.

while the secretion of extracellular serine proteases and lipase was up-regulated. Notably, α-hemolysin, an extracellular protein that was expressed at a low level in strain DB, was enhanced in the mutant and transductant A4. In contrast, exoproteins, including DNase and β-lactamase, were unaffected. Because neither DB nor 11D2 produced detectable amounts of β-hemolysin, the effect of the Tn*917*LTV1-inactivated chromosomal locus on β-hemolysin expression was also evaluated by transducing the genotype of 11D2 into the β-hemolysin-producing strain RN450. Southern blot hybridization of *Eco*RI digests of chromosomal DNA from RN450-derived transductants with a labeled *Hin*dIII fragment of pLTV1 revealed that all of the transductants hybridized to a chromosomal fragment identical in size to that of 11D2 (data not shown). Remarkably, β-hemolysin was rendered undetectable in the transductants in comparison with the parent RN450. As in 11D2, the expression of coagulase in RN450 transductants was diminished (titer 1:4) when compared with the parent (titer 1:16).

The alteration in cell-bound proteins was also studied. Expression of fibrinogen- and fibronectin-binding proteins was diminished in 11D2 and A4. Transductants from RN450 also demonstrated a marked decrease in the expression of fibrinogen-binding protein (as determined by cpm of ^{125}I-fibrinogen bound, which was 1614 ± 535 for a transductant vs. 5205 ± 344 in parent strain RN450).

Extracellular protein A was titered by a capture ELISA with immobilized human IgG. As shown in Table 2, the amount of extracellular protein A was reduced in the mutant. In contrast, cell-wall-associated protein A of 11D2 remained unchanged as determined by immunoblots with comparable amounts of cell wall extract (Table 2). Of interest is the finding that protein A released into the culture medium from mutant 11D2 and transductant A4 appeared to have a lower molecular mass (31 kDa) compared with that of strain DB (54 kDa) (data not shown).

Genetic Evidence That the Chromosomal Locus Inactivated by a Single Tn*917*LTV1 Insertion in 11D2 Differs from *agr*. A plasmid, pRN6672, containing the *agr*-A21 fragment cloned in pSK265 (6) was used as a probe to determine if the chromosomal locus inactivated by Tn*917*LTV1 in 11D2 is distinct from *agr*. Except for ≈450 base pairs in the *agrA* gene, this clone encompasses almost the entire *agr* region (6). A Southern blot containing *Eco*RI and *Nco* I digests of chromosomal DNA from DB, 11D2, and several transductants was allowed to hybridize with a ^{32}P-labeled *agr* probe and then reprobed with a ^{32}P-labeled *Hin*dIII fragment of Tn*917*LTV1 after removal of the *agr* probe. As displayed in Fig. 5, the *agr* probe hybridized to a fragment distinct from that of Tn*917*LTV1 insertion in 11D2 and transductants in both Southern blots. In addition, the restriction fragment of DB that hybridized to the *agr* probe corresponded to the fragments detected in the mutant and transductants. These data show that the chromosomal locus inactivated in 11D2 differs from *agr*.

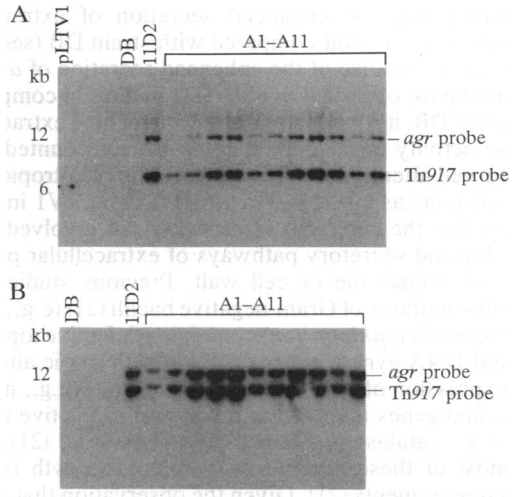

FIG. 5. Southern blots of chromosomal DNA of parent DB, mutant 11D2, and transductants A1–A11 digested with *Nco* I (*A*) or *Eco*RI (*B*). The blot was probed with a ^{32}P-labeled *agr* probe (pRN6672), stripped of the probe in a boiling solution of 0.5% SDS for 10 min, and reprobed with a 1.5-kb *Hin*dIII fragment of Tn*917*LTV1 (see text). The two autoradiograms with identical restriction digests were then superimposed to facilitate interpretation.

DISCUSSION

Insertion of Tn*917*LTV1 into a single site of the *S. aureus* chromosome of strain DB, a wild-type blood isolate, resulted in the alteration of expression of several extracellular and cell-wall-associated proteins. Southern blot hybridization with both Tn*917*LTV1 and *agr* probes (Fig. 5) indicated that the location of Tn*917*LTV1 insertion into mutant 11D2 was distinct from *agr*. Transduction experiments revealed that the phenotype associated with the genotype in 11D2 could be transferred into parent DB as well as RN450. This suggests that Tn*917*LTV1 was probably inserted into a locus involved in the regulation of a variety of extracellular and cell wall proteins.

In addition to genetic evidence, detailed phenotypic comparison between mutant 11D2 and an *agr*-defective mutant and their corresponding parental strains provided confirmation that we had identified a chromosomal locus, designated *sar*, that was inactivated by Tn*917*LTV1 in 11D2 and is distinct from *agr* (Table 2) (5, 6). In particular, the expression of α-hemolysin, which is not detectable in Agr$^-$ mutants, was enhanced in mutant 11D2 while it remained at a low level in parent DB (Table 2). Likewise, secretion of exoproteins such as serine protease and lipase was enhanced in the mutant 11D2 compared with DB, while the proteins either were rendered undetectable or were significantly decreased in the Agr$^-$ mutant. The expression of β-hemolysin, which was substantially reduced in Agr$^-$ mutants, was shown to be undetectable when the *sar* mutation was transduced into RN450, a β-hemolysin producer with a genetic background similar to strain 8325-4. However, exoproteins such as DNase, which was unaffected in the 11D2 mutant, were expressed at high levels in Agr$^+$ strains and low to moderate levels in Agr$^-$ mutants (6). Of note is the fact that the pattern of expression of δ-hemolysin from strain DB to mutant 11D2 appeared to be similar to that found in the conversion of Agr$^+$ to Agr$^-$ phenotypes.

With the exception of cell-wall-associated protein A, most of the cell-bound proteins examined were expressed at lower levels in the mutant than the parent. This pattern is contrary to that found in switching from Agr$^+$ to Agr$^-$ phenotypes (4, 6). There was also a decrease in the secretion of extracellular protein A in Sar$^-$ mutants. In addition, immunoblots revealed a lower molecular weight protein A in the mutant 11D2 compared with strain DB. One plausible explanation for this discrepancy may be enhanced secretion of extracellular proteases in the mutant compared with strain DB (see Table 2). However, because of the enhanced secretion of α-hemolysin and lipase observed in the 11D2 mutant in comparison with parent DB, it is unlikely that the augmented extracellular protease activity in the mutant could have accounted for all the observed alteration in phenotypes. The pleiotropic effect on exoproteins as a result of a single Tn*917*LTV1 insertion suggests that the *sar* locus is probably not involved in the processing and secretory pathways of extracellular proteins in the cell membrane or cell wall. Previous studies have shown that mutants of Gram-negative bacilli (21) (e.g., *E. coli* and *Salmonella typhimurium*) containing defective topoisomerase and DNA gyrase genes exhibit a pleiotropic alteration in the expression of catabolite-sensitive genes (e.g., maltose operon) and genes involved in repair and protective mechanisms (e.g., catalase and superoxide dismutase) (21). However, most of these mutants have altered growth rates or growth requirements (21). Given the observation that mutant 11D2 and parent DB have similar growth rates, it appears unlikely that the *sar* locus is implicated in either the topoisomerase or the DNA gyrase activity.

The postexponential regulation of exoproteins genes in *S. aureus* involves at least one well-characterized global regulatory system, *agr*, which affects the expression of a large number of extracellular proteins (6). The *agr* locus is probably the site of other pleiotropic exoprotein mutants (Exp$^-$) (5, 6). Although RNA III, a transcript of *agr* that acts as a regulatory molecule at the transcriptional level (6), is found to be necessary for the regulation of postexponential phase exoprotein synthesis, it has been speculated that additional global regulatory components may be involved in the control of exoprotein expression (6). For instance, strain RN4220, which has an unknown *agr* defect (6), synthesizes RNA III at nearly the same level as wild-type Agr$^+$ strains (6) but expresses hemolysins and other exoproteins only weakly. Whether the staphylococcal accessory regulator, *sar*, identified in the mutant 11D2 interacts directly or indirectly with *agr* in the regulation of target cell wall and exoprotein genes is not clear. A careful comparison of the phenotypic differences between Sar$^-$ (i.e., 11D2) and Agr$^-$ mutants in comparison with their parents revealed that with the exception of δ-hemolysin, the effect of inactivating the *sar* locus on exoprotein expression is diametrically opposed to that found in *agr*$^-$ deletion mutants. Thus, it is conceivable, yet unproven, that the *sar* locus may act as a counter-regulatory system to that of *agr*. Future analysis of the DNA sequence and transcription of the *sar* locus would shed light on the mechanism of action of this gene(s), which is involved in the regulation of extracellular and cell wall proteins in *S. aureus*.

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