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Differential Roles of Poly-*N*-Acetylglucosamine Surface Polysaccharide and Extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* Biofilms[▽]

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***Staphylococcus aureus* and *Staphylococcus epidermidis* are major human pathogens of increasing importance due to the dissemination of antibiotic-resistant strains. Evidence suggests that the ability to form matrix-encased biofilms contributes to the pathogenesis of *S. aureus* and *S. epidermidis*. In this study, we investigated the functions of two staphylococcal biofilm matrix polymers: poly-*N*-acetylglucosamine surface polysaccharide (PNAG) and extracellular DNA (ecDNA). We measured the ability of a PNAG-degrading enzyme (dispersin B) and DNase I to inhibit biofilm formation, detach preformed biofilms, and sensitize biofilms to killing by the cationic detergent cetylpyridinium chloride (CPC) in a 96-well microtiter plate assay. When added to growth medium, both dispersin B and DNase I inhibited biofilm formation by both *S. aureus* and *S. epidermidis*. Dispersin B detached preformed *S. epidermidis* biofilms but not *S. aureus* biofilms, whereas DNase I detached *S. aureus* biofilms but not *S. epidermidis* biofilms. Similarly, dispersin B sensitized *S. epidermidis* biofilms to CPC killing, whereas DNase I sensitized *S. aureus* biofilms to CPC killing. We concluded that PNAG and ecDNA play fundamentally different structural roles in *S. aureus* and *S. epidermidis* biofilms.**

Staphylococcus aureus and *Staphylococcus epidermidis* are among the most common bacteria isolated from human infections. *S. aureus* colonizes mucosal surfaces such as the anterior nares, whereas *S. epidermidis* is part of the normal microflora of the skin. Infection results when a breach in the mucosal barrier or skin allows bacterial cells access to the underlying tissues or to the bloodstream (36, 39). *S. aureus* causes numerous infections, ranging from acute skin abscesses to life-threatening bacteremias and endocarditis (36). *S. epidermidis* is a leading cause of infections associated with implanted medical devices (56). The increasing incidence of antibiotic-resistant strains of *S. aureus* and *S. epidermidis* has heightened efforts to find new ways to fight these pathogens (6, 52).

Both *S. aureus* and *S. epidermidis* are known for their ability to form biofilms, which are defined as communities of bacteria, encased in a self-synthesized extracellular polymeric matrix, growing attached to a biotic or abiotic surface (15, 19, 42). Biofilms that form on tissues or medical devices are extremely difficult to eradicate because the biofilm mode of growth protects bacterial cells from killing by antibiotics and host defenses (18). Evidence suggests that biofilm formation plays a role in *S. aureus* wound infections (2) and osteomyelitis (7) and in *S. epidermidis* catheter infections (10).

Numerous studies have identified and characterized extracellular factors that mediate surface attachment and intercellular adhesion in *S. aureus* and *S. epidermidis* biofilms. Both species produce poly-β(1,6)-*N*-acetyl-D-glucosamine (PNAG), a surface polysaccharide that is sometimes referred to as polysaccharide intercellular adhesin (11, 37). PNAG contributes to biofilm accumulation and immune evasion in both *S. aureus* and *S. epidermidis* (10, 35, 57). However, not all strains produce PNAG, and many PNAG-deficient strains exhibit a strong biofilm phenotype (42, 48, 54). Recently, extracellular DNA (ecDNA) has also been shown to comprise a structural component of the *S. aureus* and *S. epidermidis* biofilm matrix (13, 46, 47). In addition, proteinaceous adhesins, such as *S. aureus* biofilm-associated protein (12) and *S. epidermidis* accumulation-associated protein (24), as well as cell wall teichoic acids (20, 50, 55), have been shown to participate in surface attachment and biofilm cohesion.

The purpose of the present study was to gain better insight into the functional roles of PNAG and ecDNA in *S. aureus* and *S. epidermidis* biofilms. By treating biofilms grown in 96-well microtiter plates with a PNAG-degrading enzyme (dispersin B) and DNase I, we obtained evidence that PNAG and ecDNA perform markedly different functions in *S. aureus* and *S. epidermidis* biofilms.

MATERIALS AND METHODS

Reagents. Bovine DNase I, cetylpyridinium chloride (CPC) (hexadecylpyridinium chloride), sodium dodecyl sulfate (SDS), and phosphate-buffered saline (PBS) were purchased from Sigma Chemical Co. (St. Louis, MO). Restriction endonucleases were purchased from Invitrogen (Carlsbad, CA). Dispersin B (31) was obtained from Kane Biotech, Inc. (Winnipeg, Canada). Gram's crystal violet stain (no. 23255960) was purchased from Fisher Scientific (Fair Lawn, NJ).

Bacterial strains, media, and growth conditions. The bacterial strains used in this study were *S. aureus* SH1000 (23), 8325 (41), MRSA252 (22), MZ100 (51), and ATCC 6341 (American Type Culture Collection, Manassas, VA), as well as *S. epidermidis* NJ9709 (32) and 1467 (38). All strains were passaged weekly on blood agar and stored at 4°C. Biofilms were cultured in tryptic soy broth (Becton-Dickinson, Sparks, MD) containing 6 g of yeast extract and 8 g of glucose per liter (TSB). All cultures were incubated at 37°C.

Biofilm formation assay. A loopful of cells from an agar plate was transferred to a polypropylene microcentrifuge tube containing 200 μl of TSB. The cells were crushed with a disposable pellet pestle, vortexed for 30 s, diluted to 1 ml in fresh TSB, and then passed through a 5-μm-pore-size syringe filter to remove large clumps of cells, as previously described (30). Filtered cells were diluted to 10^3 to 10^5 CFU/ml in TSB. For inhibition studies, filtered cells were diluted in

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TSB containing 20 μg/ml of dispersin B or 100 μg/ml of DNase I. Aliquots of cells (200 μl each) were transferred to the wells of a 96-well tissue-culture-treated polystyrene microtiter plate (Falcon no. 324662; Becton-Dickinson), and the plate was incubated for 24 h. Biofilms were washed once with water and then dried. Biofilms were stained for 1 min with 200 μl of Gram's crystal violet and then rinsed with water and dried. The amount of biofilm biomass was quantitated by destaining the biofilms for 10 min with 33% acetic acid (by vol) and then measuring the absorbance of the crystal violet solution at 590 nm (A_{590}).

Biofilm detachment assay. Biofilms were grown in 96-well microtiter plates as described above. Biofilms were rinsed once with water and then treated with 200 μl of dispersin B (20 μg/ml in PBS), DNase I (100 μg/ml in 150 mM NaCl, 1 mM $CaCl_2$), or various restriction endonucleases (20 U/ml in 10 mM Tris [pH 7.9], 10 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol). Control wells were treated with 200 μl of the appropriate buffer alone. After 1 h at 37°C, biofilms were rinsed with water and stained with crystal violet as described above.

Biofilm killing assay. Biofilms were grown in 96-well microtiter plates as described above. Biofilms were rinsed once with water and then treated with 200 μl of TSB containing 20 μg/ml of dispersin B or 100 μg/ml of DNase I. Control wells were treated with 200 μl of TSB alone. Control experiments indicated that dispersin B and DNase I did not affect *S. aureus* or *S. epidermidis* growth and viability (data not shown). After 10 min at 37°C, 20 μl of 3% CPC (for *S. aureus*) or 1% CPC (for *S. epidermidis*) was added to each well and biofilms were incubated for 5 min at room temperature. Control wells received 20 μl of water. For biofilms treated with TSB alone and for *S. aureus* biofilms treated with dispersin B and *S. epidermidis* biofilms treated with DNase I, biofilms were washed four times with PBS to remove the CPC and then treated with 100 μg/ml of DNase I (for *S. aureus*) or 20 μg/ml of dispersin B (for *S. epidermidis*) to dissolve the biofilm. These reactions were carried out in the enzyme buffers described above. After 10 min, cells were mixed and then serial dilutions were plated on agar. For *S. aureus* biofilms treated with DNase I and *S. epidermidis* biofilms treated with dispersin B, cells were mixed and then a 50-μl aliquot of cells was diluted in 50 ml of PBS. The cells were passed through an analytical test filter funnel (no. 145-2020; Nalgene, Rochester, NY), and the filter was rinsed with 250 ml of sterile water, aseptically removed from the filter unit, and placed on a blood agar plate. Colonies were enumerated after 24 h.

Planktonic cell killing assay. Cells scraped from an agar plate were resuspended in PBS and filtered as described above. Cells were adjusted to 1×10^7 to 5×10^7 CFU/ml and transferred in 180-μl aliquots to the wells of a 96-well microtiter plate. Twenty microliters of 0.0025 to 0.01% CPC was transferred to each well. Control wells received 20 μl of water. After 5 min at room temperature, cells were diluted and plated on agar. In some experiments, planktonic cells were pretreated for 15 min with 20 μg/ml of dispersin B prior to the addition of CPC.

Total hexosamine assay. Tissue-culture-treated polystyrene petri dishes (100-mm-diameter; Falcon no. 353003) were filled with 20 ml of TSB containing 10^4 to 10^5 CFU/ml and incubated for 24 h. The resulting biofilms were rinsed with PBS and scraped into a small volume of PBS. Aliquots of cells (≈60 mg [wet weight]) were transferred to 1.5-ml polypropylene microcentrifuge tubes and resuspended in 400 μl of 20 μg/ml dispersin B in PBS. Control cells were resuspended in PBS alone. After 1 h at 37°C, cells were pelleted and the amount of total hexosamine in the supernatant was measured by using the Morgan-Elson assay as previously described (53).

Statistics and reproducibility of results. Biofilm inhibition and killing assays and biofilm detachment assays with dispersin B and DNase I were performed in duplicate wells. Biofilm detachment assays with restriction endonucleases were performed in quadruplicate wells. Replicate wells exhibited 0 to 10% variation in A_{590} or CFU/well values. The significance of differences between means was calculated using a Student *t* test. *P* values of <5% were considered significant. All inhibition and detachment assays were performed on at least three occasions with similarly significant decreases in A_{590} values. Total hexosamine assays were performed on at least two occasions with similar increases in total hexosamine release. Killing assays were performed on numerous occasions with similar highly significant decreases in CFU/well values.

RESULTS

Inhibition of biofilm formation by the PNAG-degrading enzyme dispersin B. Figure 1A shows *S. aureus* and *S. epidermidis* biofilm formation in the wells of a 96-well microtiter plate. Biofilms were stained with crystal violet, which stains bacterial cells and biofilm matrix components but not polystyrene (43). The amount of biofilm biomass was measured by destaining the biofilms and quantitating the amount of bound dye (Fig. 1B). Biofilm formation by *S. aureus* strain SH1000 and by *S. epidermidis* strains NJ9709 and 1457 was significantly inhibited in growth medium supplemented with dispersin B compared to biofilm formation in unsupplemented medium. These results are consistent with those of previous studies showing that dispersin B inhibited biofilm formation by 18 out of 18 *S. aureus* strains and 26 out of 26 PNAG-positive *S. epidermidis* strains isolated from prosthetic joint infections (48) and by *S. epidermidis* strain 1457 (25). Biofilm formation by *S. aureus* strain MRSA252, which produced weaker biofilms than strain SH1000, was not significantly inhibited by dispersin B.

Detachment of preformed biofilms by dispersin B. Fig. 1C and D show 24-h-old *S. aureus* and *S. epidermidis* biofilms treated with dispersin B. Dispersin B had no significant effect on the attachment of *S. aureus* SH1000 and MRSA252 biofilms. In contrast, dispersin B rapidly and efficiently detached *S. epidermidis* NJ9709 and 1457 biofilms. These findings are consistent with those of previous studies demonstrating that dispersin B detached biofilms produced by various strains of *S. epidermidis* (9, 25, 32) but not biofilms produced by *S. aureus* strain 383 (9).

PNAG is not a major matrix component in *S. aureus* biofilms. The amount of PNAG present in *S. aureus* and *S. epidermidis* biofilms was measured by treating biofilm cells with dispersin B and then quantitating the amount of total hexosamine in the cell supernatant using the Morgan-Elson assay (Fig. 2). Four different *S. aureus* strains did not release detectable amounts of total hexosamine when treated with dispersin B, whereas *S. epidermidis* strains NJ9709 and 1457 exhibited large increases in the amount of total hexosamine in the cell supernatant. To rule out the possibility that dispersin B does not penetrate aggregates of *S. aureus* biofilm cells, we pretreated the cells with DNase I, which disaggregates the cells (see below), prior to treating them with dispersin B. *S. aureus* biofilm cells treated in this manner still did not release detectable amounts of total hexosamine after dispersin B treatment (data not shown).

PNAG protects *S. epidermidis* biofilm cells from detergent killing. Previous studies showed that dispersin B sensitizes biofilms produced by the gram-negative bacterium *Aggregatibacter actinomycetemcomitans* to killing by the cationic detergent CPC (27). We tested whether dispersin B could also sensitize *S. aureus* SH1000 and *S. epidermidis* NJ9709 biofilms to CPC killing. Control experiments indicated that the minimal bactericidal concentration (>99.999% killing in 5 min) of CPC was 0.0003% against *S. aureus* SH1000 planktonic cells and 0.001% against *S. epidermidis* NJ9709 planktonic cells and that *S. aureus* SH1000 and *S. epidermidis* NJ9709 biofilms were resistant to killing by CPC concentrations that were 1,000-fold and 100-fold greater, respectively, than their planktonic minimal bactericidal concentrations (data not shown).

Pretreatment of *S. aureus* SH1000 biofilms with dispersin B did not increase their sensitivity to killing by 0.3% CPC in 5 min (Fig. 3A). In contrast, pretreatment of *S. epidermidis* NJ9709 biofilms with dispersin B resulted in a 5- to 6-log unit decrease in CFU/well values after treatment with 0.1% CPC for 5 min compared to values for control biofilms that were pretreated with PBS alone. Time course studies showed that killing of dispersin B-treated *S. epidermidis* biofilms by CPC

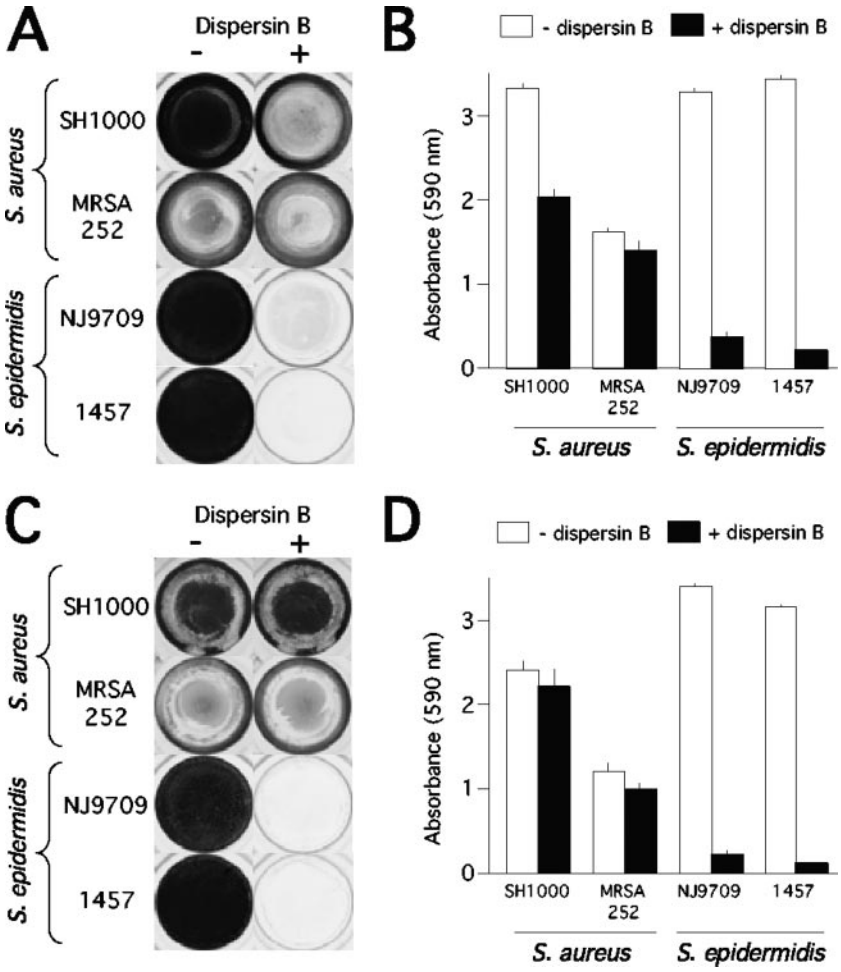

FIG. 1. Inhibition and detachment of *S. aureus* and *S. epidermidis* biofilms by dispersin B in 96-well microtiter plates. (A) The indicated strains were grown for 24 h in unsupplemented TSB medium (−) or TSB supplemented with 20 μg/ml of dispersin B (+). Wells were rinsed and stained with crystal violet. (B) Quantitation of crystal violet staining in panel A. Wells were destained with acetic acid, and the absorbance of the crystal violet solution was measured at 590 nm. Absorbance is proportional to biofilm biomass. Values represent the means for duplicate wells, and error bars indicate range. (C) Biofilms were grown for 24 h in unsupplemented TSB and then rinsed, treated for 1 h with PBS (−) or PBS with dispersin B (+), rinsed, and stained with crystal violet. (D) Quantitation of crystal violet staining in panel C.

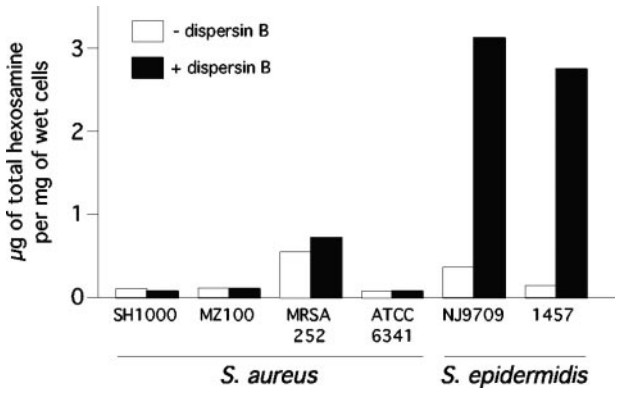

FIG. 2. Quantitation of total hexosamine released by *S. aureus* and *S. epidermidis* biofilm cells treated with dispersin B. Control cells (− dispersin B) were treated with PBS alone. Total hexosamine was measured using the Morgan-Elson assay.

was complete within 1 min (Fig. 3B). Pretreatment of *S. epidermidis* planktonic cells with dispersin B did not increase their susceptibility to killing by CPC (Fig. 3C), indicating that PNAG protects *S. epidermidis* cells from CPC killing at the multicellular level.

Inhibition of biofilm formation by DNase I. Figure 4A and B show that biofilm formation by *S. aureus* strains SH1000 and MRSA252 and by *S. epidermidis* strain 1457 was significantly inhibited in medium supplemented with DNase I. These findings are consistent with those of previous studies demonstrating that DNase I inhibits biofilm formation by two different *S. aureus* clinical isolates (13, 47) and six different *S. epidermidis* reference strains and clinical isolates (46).

Detachment of biofilms by DNase I. Figure 4C and D show 24-h-old *S. aureus* and *S. epidermidis* biofilms treated for 1 h with DNase I. DNase I caused significant detachment of *S. aureus* biofilms from the surface. Microscopic analysis indicated that DNase I rapidly dispersed *S. aureus* biofilms into uniform suspensions of small cell clusters which did not dis-

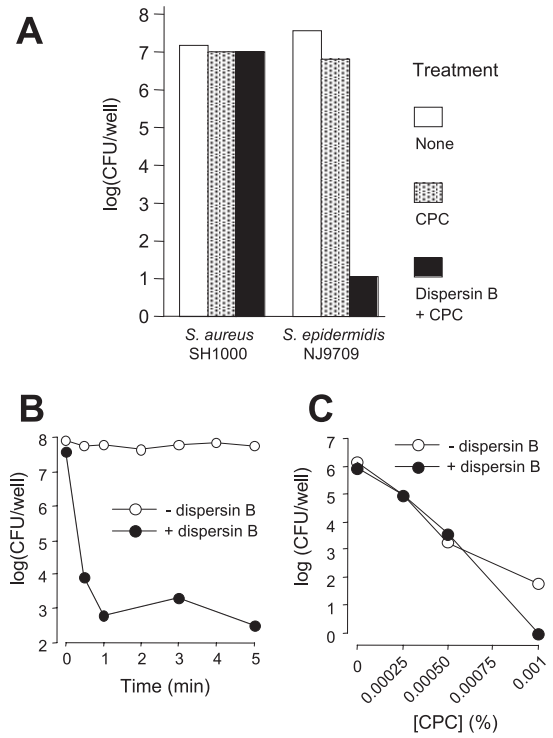

FIG. 3. Pretreatment of *S. epidermidis* biofilms with dispersin B renders them sensitive to killing by CPC. (A) *S. aureus* SH1000 and *S. epidermidis* NJ9709 biofilms grown in 96-well microtiter plates were treated with 0.3% CPC (for *S. aureus*) or 0.1% CPC (for *S. epidermidis*) for 5 min. Values show mean numbers of surviving CFU/well, and error bars indicate range. Black bars show CFU/well values for biofilms pretreated with 20 μg/ml of dispersin B for 10 min prior to addition of the CPC. (B) Time course for CPC killing of *S. epidermidis* NJ9709 biofilms with or without dispersin B pretreatment. (C) Five-minute killing of *S. epidermidis* NJ9709 planktonic cells by CPC with or without dispersin B pretreatment.

perse into single cells upon extended incubation (data not shown). DNase I did not cause significant detachment of biofilms produced by *S. epidermidis* 1457 or NJ9709. These findings are consistent with those of previous studies demonstrating that mature biofilms produced by six different *S. epidermidis* reference strains and clinical isolates were resistant to detachment by DNase I (46).

To confirm that ecDNA comprises a structural component of the *S. epidermidis* biofilm matrix, we treated *S. epidermidis* NJ9709 biofilms with DNase I and then with a 1% solution of the anionic detergent SDS (Fig. 5). Previous studies showed that *Pseudomonas aeruginosa* biofilms pretreated with DNase I were sensitized to detachment by SDS (3). DNase I pretreatment did not sensitize *S. epidermidis* biofilms to SDS detachment. However, SDS caused DNase I-treated *S. epidermidis* biofilms to condense into long bundles of thick fibrils that covered the entire surface of the well (Fig. 5). Neither DNase I nor SDS had any effect on the viability of *S. epidermidis* cells under these conditions (data not shown).

Detachment of *S. aureus* biofilms by restriction endonucleases. We tested the ability of various restriction endonucleases to detach *S. aureus* SH1000 biofilms (Fig. 6A). The amount of biofilm detachment depended on the frequency of the enzyme recognition sequence in the *S. aureus* genome (Fig. 6A). Enzymes that produced restriction fragments with an average size of <10 kb caused efficient biofilm detachment, whereas enzymes that produced restriction fragments with an average size of 11 to 24 kb caused partial detachment. These findings suggest that the fraction of *S. aureus* ecDNA that mediates intercellular adhesion is composed primarily of genomic DNA. Biofilms produced by three other *S. aureus* strains (MZ100, MSRA252, and 8325) exhibited similar detachment phenotypes when treated with HinfI (average restriction fragment size, 0.4 kb) or BamHI (average restriction fragment size, 23.9 kb) (Fig. 6B).

ecDNA protects *S. aureus* biofilm cells from detergent killing. We tested the ability of DNase I to render *S. aureus* SH1000 and *S. epidermidis* NJ9709 biofilm cells sensitive to killing by CPC (Fig. 7). Pretreatment of *S. aureus* SH1000 biofilms with DNase I resulted in a 4-log-unit decrease in CFU/well values after treatment with 0.3% CPC for 5 min compared to results for control biofilms that were pretreated with DNase I buffer alone. DNase I had an identical effect on killing of *S. aureus* MRSA252 and 8325 biofilm cells by CPC (data not shown). DNase I did not sensitize *S. epidermidis* NJ9709 biofilms to killing by 0.01% CPC in 5 min (Fig. 7).

DISCUSSION

Biochemical studies have confirmed that PNAG is produced by both *S. aureus* and *S. epidermidis* (29, 37). Homologues of the *icaADBC* locus, which encodes the production of PNAG in *S. aureus* and *S. epidermidis*, have been identified in various other staphylococci, including *S. caprae*, *S. capitis*, *S. cohnii*, *S. lugdunensis*, *S. pasteuri*, and *S. saprophyticus* (4, 16, 17, 40). However, PNAG production has not been directly demonstrated in these species. A polysaccharide that is nearly identical to staphylococcal PNAG is produced by various gram-negative *Proteobacteria*, including *Escherichia coli*, *Pseudomonas fluorescens*, *Yersinia pestis*, *Actinobacillus pleuropneumoniae*, *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, and *Bordetella* spp. (25, 33, 44). Functions ascribed to proteobacterial PNAG include abiotic surface attachment, intercellular adhesion, biofilm formation, detergent resistance, antibiotic resistance, and epithelial cell attachment (1, 25–27, 45).

Several observations suggest that PNAG performs different functions in *S. aureus* and *S. epidermidis* biofilms. For example, *S. aureus* PNAG mutants can still form biofilms in vitro and in vivo, whereas *S. epidermidis* PNAG mutants exhibit a severely reduced biofilm phenotype (5, 21). Also, nearly all strains of *S. aureus* carry the *icaADBC* locus (42), whereas *ica* genes are absent in up to half of *S. epidermidis* strains isolated from device-associated infections (8) and in nearly all skin and mucosal isolates (58). In addition, *S. epidermidis* mutant strains deficient in PNAG production exhibit decreased virulence in animal models of both systemic and device-associated infection, whereas *S. aureus* PNAG-deficient strains exhibit reduced virulence in animal models of systemic but not device-associated infection (34, 35, 49). In the present study, we showed that the PNAG-degrading enzyme dispersin B was able to detach preformed biofilms produced by *S. epidermidis* and render them sensitive to detergent killing whereas dispersin B had no effect on the attachment of preformed *S. aureus* biofilms or

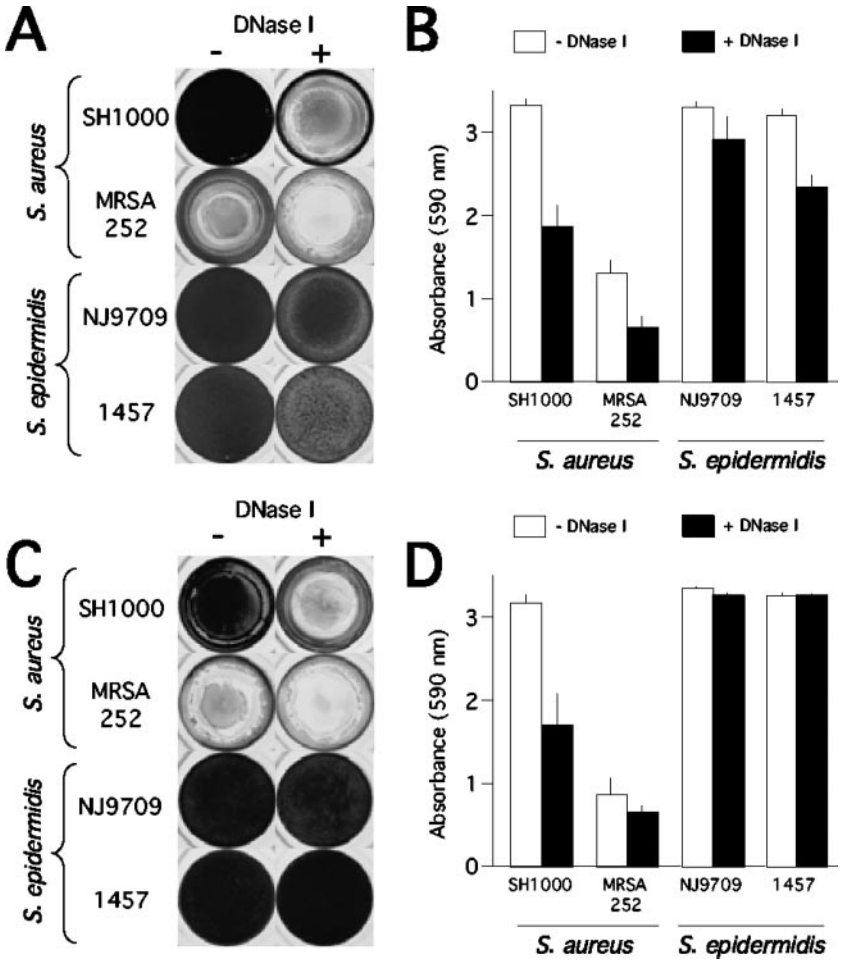

FIG. 4. Inhibition and detachment of *S. aureus* and *S. epidermidis* biofilms by DNase I in 96-well microtiter plates. (A) The indicated strains were grown for 24 h in unsupplemented TSB medium (−) or TSB supplemented with 100 μg/ml of DNase I (+). Wells were rinsed and stained with crystal violet. (B) Quantitation of crystal violet staining in panel A as described in the legend to Fig. 1. (C) Biofilms were grown for 24 h in unsupplemented TSB and then rinsed, treated for 1 h with DNase I buffer alone (−) or DNase I buffer with DNase I (+), rinsed, and stained with crystal violet. (D) Quantitation of crystal violet staining shown in panel C.

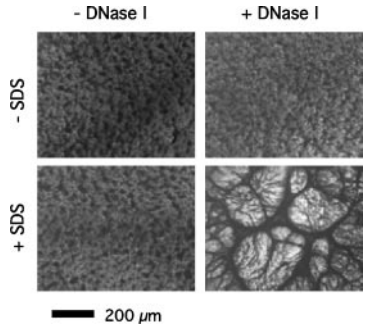

FIG. 5. Close-up view of 24-h-old *S. epidermidis* NJ9709 biofilms in 96-well microtiter plates. Top left, untreated biofilm; top right, biofilm treated with 100 μg/ml of DNase I for 1 h; bottom left, biofilm treated with 1% SDS for 5 min; bottom right, biofilm treated with DNase I for 1 h and then SDS for 5 min. Biofilms were stained with crystal violet and then viewed and photographed under an Olympus IMT-2 inverted microscope at magnification ×40.

their sensitivity to detergent killing (Fig. 1C and 3). In addition, only small quantities of hexosamine were released from *S. aureus* biofilms treated with dispersin B compared to the amount released by *S. epidermidis* biofilms (Fig. 2). Taken together, these findings suggest that PNAG is a major matrix adhesin in *S. epidermidis* biofilms and a minor component of *S. aureus* biofilms. The role of PNAG in *S. aureus* biofilms may be similar to its role in *S. lugdunensis* biofilms, which are also resistant to detachment by dispersin B (17).

When added to the culture medium at the time of inoculation, dispersin B inhibited biofilm formation by *S. aureus* strain SH1000 (Fig. 1A). The fact that mature *S. aureus* biofilms were resistant to detachment by dispersin B suggests that PNAG may function in the early stages of *S. aureus* biofilm development.

In the present study, we also examined the role of ecDNA in *S. aureus* and *S. epidermidis* biofilm formation. Our data confirm that ecDNA is a structural component of the biofilm matrix in both species (Fig. 4 and 5) (13, 46, 47). However, our

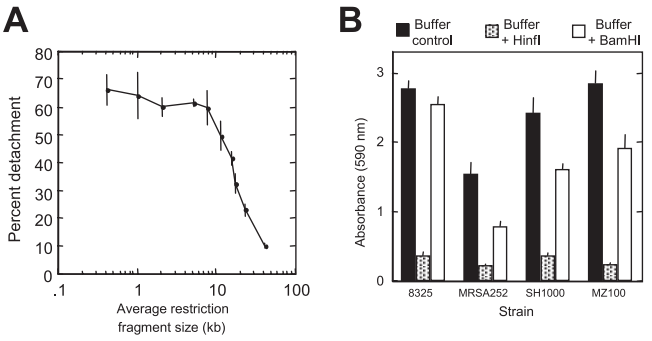

FIG. 6. Detachment of *S. aureus* biofilms by restriction endonucleases. (A) *S. aureus* SH1000 biofilms grown in microtiter plates were treated for 1 h with 100 U/ml of various restriction endonucleases and then rinsed and stained with crystal violet. Percent detachment was calculated as $1 - (A_{595}[\text{buffer + enzyme}]/A_{595}[\text{buffer alone}]) \times 100$. Values show means and ranges for duplicate wells. The restriction endonucleases used (and average restriction fragment lengths in kilobases) were HinfI (0.4), FokI (1.0), HaeIII (2.1), AlwNI (5.1), ApaLI (7.8), NcoI (11.8), KpnI (16.2), AvaI (17.9), BamHI (23.9), and SstI (41.5). (B) Detachment of *S. aureus* 8325, MRSA252, SH1000, and MZ100 biofilms by HinfI and BamHI. Biofilms were treated with 100 U/ml of enzyme for 1 h, and then biofilm biomass was quantitated by crystal violet staining. Values show means and standard errors for quadruplicate wells.

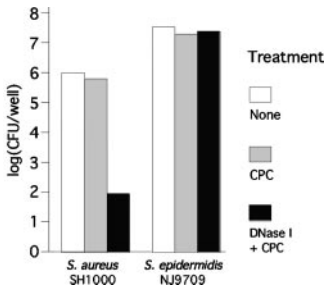

FIG. 7. Pretreatment of *S. aureus* biofilms with DNase I renders them sensitive to killing by CPC. (A) *S. aureus* SH1000 and *S. epidermidis* NJ9709 biofilms grown in microtiter plates were treated with 0.3% CPC (for *S. aureus*) or 0.1% CPC (for *S. epidermidis*) for 5 min. Values show mean numbers of surviving CFU/well for duplicate wells. Black bars show CFU/well values for biofilms pretreated with 100 μg/ml of DNase I for 10 min prior to addition of the CPC.

findings suggest that ecDNA is a major structural component in the *S. aureus* biofilm matrix and a minor structural component in the *S. epidermidis* biofilm matrix. This hypothesis is based on the fact that DNase I inhibited *S. aureus* biofilm formation, detached preformed *S. aureus* biofilms, and rendered preformed *S. aureus* biofilms sensitive to detergent killing whereas DNase I had no effect on *S. epidermidis* biofilm formation, attachment, or detergent sensitivity (Fig. 4 and 7). Previous studies showed that *S. aureus* ecDNA may be comprised of genomic DNA released from lysed biofilm cells (47). Consistent with this hypothesis, we found that the ability of various restriction endonucleases to detach preformed *S. aureus* biofilms depended on the frequency of their recognition sequence in the *S. aureus* genome (Fig. 6). The results of these studies suggest that *S. aureus* ecDNA fragments of >11 kb in size can function as intercellular adhesins. Interestingly, we found that micrococcal nuclease rapidly and efficiently detached *S. aureus* biofilms (unpublished data), suggesting that this enzyme may play a role in biofilm cell detachment and biofilm dispersal.

Our findings indicate that depolymerization of either *S. aureus* ecDNA or *S. epidermidis* PNAG sensitizes the respective biofilm cells to killing by the cationic detergent CPC (Fig. 3 and 7). Previous studies showed that depolymerization of PNAG sensitizes *A. actinomycetemcomitans* biofilm cells to killing by the anionic detergent SDS (28). In all cases, detergent sensitivity is associated with dissolution and detachment of the biofilm cells. Taken together, these findings suggest that matrix polymers such as ecDNA and PNAG can act as general diffusion barriers that prevent access of detergents to the biofilm cells. However, previous studies also showed that exogenously added *S. epidermidis* PNAG can interfere with the antimicrobial activity of glycopeptide antibiotics, including vancomycin and teicoplanin (14). These findings suggest that PNAG may also form physical complexes with some antimicrobial agents, thereby sequestering the agents within the polymeric matrix.

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