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Detection of the Intercellular Adhesion Gene Cluster (*ica*) and Phase Variation in *Staphylococcus epidermidis* Blood Culture Strains and Mucosal Isolates

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***Staphylococcus epidermidis* is a common cause of catheter-associated infections and septicemia in immunocompromised patients. To answer the question whether *S. epidermidis* skin isolates differ from isolates causing septicemic diseases, 51 strains obtained from blood cultures, 1 strain from shunt-associated meningitis, and 36 saprophytic isolates were characterized. The study demonstrates that most of the blood culture strains formed a multilayered biofilm on plastic material, whereas skin and mucosal isolates did not. Moreover, biofilm-producing strains were found to generate large bacterial autoaggregates in liquid culture. Autoaggregation and biofilm formation on polymer surfaces was associated with the presence of a DNA sequence encoding an intercellular adhesion gene cluster (*ica*) that mediates the production of a polysaccharide intercellular adhesin. The presence of the intercellular adhesion genes in blood culture isolates was also found to be correlated with the exhibition of black colonies on Congo red agar, whereas the adhesin-negative strains formed red colonies. Upon subcultivation on Congo red agar, the black colony forms of the blood culture strains exhibited red colony variants which were biofilm and autoaggregation negative and occurred at a frequency of 10^{-5}. The DNA analysis of these *S. epidermidis* variants by pulsed-field gel electrophoresis and Southern hybridization with an *ica*-specific gene probe revealed no detectable difference between the black and red colony types. Moreover, after repeated passage, the phenotype of the parent strain could be restored. Therefore, these colony forms were regarded as phase variants. This phenotypic change was observed exclusively in adhesin-positive clinical isolates and not in adhesin-negative saprophytic strains of *S. epidermidis*.**

Coagulase-negative staphylococci, e.g., *Staphylococcus epidermidis*, are known to be part of the normal skin and mucosal microflora. In recent years, however, *S. epidermidis* emerged as a common cause of nosocomial infections in immunocompromised patients (23, 33). Septicemia caused by *S. epidermidis* is often associated with the use of intravenous catheters and other medical devices. An additional problem of these infectious diseases is the increasing resistance of staphylococci to oxacillin and other antibiotics and the spread of multiresistant isolates within the hospital environment (6). There is a strong correlation between the use of indwelling medical devices and the emergence of staphylococcal infections. However, the information on the microbial mechanisms and factors contributing to the virulence of *S. epidermidis* is limited.

It has been proposed that the staphylococcus adherence to polymer surfaces contributes considerably to the pathogenesis of polymer-associated infections (7, 31, 32). Recent data suggest that the bacterial biofilm is produced in a two-step manner, i.e., the initial bacterial monolayer is converted to a typical biofilm consisting of bacteria and an extracellular slime substance (20). Both polysaccharide adhesins (10, 27, 28, 29, 40) and protein components (39) that are involved in initial adherence as well as in biofilm accumulation have been described. Recently, mutants with altered initial adherence and autoaggregation properties have been generated by chemical and transposon mutagenesis (20, 35). The data obtained from these experiments indicate that initial adherence and biofilm formation by intercellular adhesion and slime production are distinct events. In the initial binding of *S. epidermidis*, a 60-kDa surface-associated protein is involved (20), whereas cell aggregation and biofilm accumulation were found to be mediated by the products of a gene locus comprising three intercellular adhesion genes (*icaABC*) which are organized in an operon structure (21). It was shown that the *ica*-encoded genes lead to the biosynthesis of the polysaccharide intercellular adhesin (PIA) (21), which contains *N*-acetylglucosamine as a major component and is involved in the accumulative stage of biofilm formation.

Another important property of staphylococci is their capacity to change specific phenotypic features rapidly. Several studies showed that adherence, slime production, and resistance to antibiotics undergo changes and differ among variants of the same parent strain (2, 3, 9, 11–13, 30). In this context, it has been suggested that phase variation may represent a virulence factor contributing to bacterial survival and growth under changing environmental conditions.

The question of whether differences between *S. epidermidis* strains causing infections and those isolated from healthy skin and mucosal flora exist is of central importance to the understanding of *S. epidermidis* pathogenesis. To address this question, we compared, in this study, strains isolated from patients with plastic material-associated infections with isolates from skin and mucosa. Our data indicate that *S. epidermidis* strains from clinical material differ from saprophytic strains in terms of the presence of the *ica* gene cluster as well as in the capac-

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TABLE 1. Properties of *S. epidermidis* blood culture and saprophytic isolates[a]

Type of isolates (*n*)	No. (%) of isolates					
	Biofilm positive	Biofilm negative	Autoaggregated in TSB	With *ica* genes	Colony morphology on CRA	
					Black	Red
Blood culture (52)	45[b] (87)	7[c] (13)	41 (79)	44 (85)	45 (87)	7 (13)
Saprophytic (36)	4[d] (11)	32[c] (89)	2 (6)	2 (6)	2 (6)	34 (94)

[a] Biofilm formation was detected by the quantitative adherence assay described in Materials and Methods. Autoaggregation was detected by Southern hybridization.
[b] This total number included 3 isolates with an OD_{490} of >0.12 but <0.60, 30 isolates with an OD_{490} of >0.60 but <2.00, and 12 isolates with an OD_{490} of >2.00.
[c] All of these isolates had an OD_{490} of <0.12.
[d] This total number included three isolates with an OD_{490} of >0.12 but <0.60 and one isolate with an OD_{490} of >0.60.

ities for phase variation, polymer adherence, autoaggregation, and colony morphology on Congo red agar (CRA).

MATERIALS AND METHODS

Bacterial strains. Fifty-one *S. epidermidis* strains obtained from a blood culture strain collection, one *S. epidermidis* strain isolated from cerebrospinal fluid, and 36 skin and mucosal isolates from healthy volunteers were investigated. Skin and mucosal strains were isolated by swabbing either the anterior nares or the nondominating hand of the test person, respectively. Staphylococci were identified by colonial appearance on whole blood agar (5% defibrinated human erythrocytes) and Gram's stain.

Biotyping and antibiotic susceptibility. Species determination and biotyping were performed by use of a numeric profile based upon 20 biochemical reactions as described in the manufacturer's instructions (API-Staph; BioMerieux, Nürtingen, Germany). Antibiotic susceptibility was tested by the standard agar diffusion method on Mueller-Hinton agar plates supplemented with 5% sodium chloride.

Colony morphology and detection of phenotypic variants. Colony morphology and its phenotypic change were studied on CRA, which was prepared by adding 0.8 g of Congo red (Serva, Heidelberg, Germany) and 36 g of saccharose (Roth, Karlsruhe, Germany), both of which had been previously autoclaved separately, to 1 liter of brain heart infusion agar (Oxoid, Basingstoke, England) (14). Plates were incubated for 24 h at 37°C and subsequently overnight at room temperature. Colony morphology was examined with a plate microscope. The variation rate was determined by counting the different colony forms in relation to the total number of CFU.

Initial adherence on polymer surfaces. The assay for the determination of the initial adherence was essentially performed as described previously (20). Briefly, bacterial cultures were grown in Trypticase soy broth (TSB) at 37°C to the early stationary phase. Cultures were diluted with sterile phosphate-buffered saline (PBS) to an optical density at 600 nm (OD_{600}) of 0.1. A polystyrene petri dish (Sarstedt, Nürnbrecht, Germany) was filled with an aliquot of 10 ml and incubated at 37°C for 30 min. After extensive washing of the dishes with PBS, the adhered cells were visualized by microscopy, photographed, and counted.

Biofilm formation and autoaggregation. Quantitative estimations of biofilm formation were performed by the method of Christensen et al. (8) with the following modification. TSB with the usual glucose supplementation (Difco Laboratories, Detroit, Mich.) was used exclusively. For each strain, the test was performed eight times in a tissue culture plate (Greiner, Nürtingen, Germany). Following washing and fixation as described previously (8), adherent biofilm was stained for 10 min with 0.4% crystal violet solution. The absorbance at 490 nm was determined. The well-characterized biofilm-producing strain *S. epidermidis* RP62A (ATCC 35984) and the biofilm-negative *Staphylococcus carnosus* TM 300 strain were used as positive and negative controls, respectively. An OD_{490} of >0.120 was regarded as biofilm positive.

For autoaggregation screening, strains were cultivated in tissue culture plates overnight at 37°C in TSB and examined by light microscopy.

Detection of PIA by immunofluorescence microscopy. Immunofluorescence microscopy was performed essentially as described previously (27), with minor modifications. Cultures were grown in TSB overnight at 37°C. Aliquots of 20 μl were applied to slides and air dried. Following fixation by heat, the slides were incubated with PIA-specific antiserum diluted 1:100 in PBS for 45 min at room temperature. The PIA-specific antiserum had been absorbed before with an *ica*-negative *S. epidermidis* strain as described previously (27). Thereafter, the slides were washed three times with PBS and incubated with Texas red-conjugated goat anti-rabbit immunoglobulin G antibody (Dianova, Hamburg, Germany) diluted 1:150 in PBS for 45 min at room temperature. The slides were washed three times in PBS and once in distilled water. After air drying, the slides were embedded in Citifluor and viewed with a fluorescence microscope (Zeiss, Oberkochen, Germany).

Scanning electron microscopy. For scanning electron microscopy, staphylococci were grown overnight in TSB medium on polystyrene chamber slides at 37°C. After decanting the medium, the slides were washed three times with PBS, dried at 42°C for 36 h in a desiccator, mounted on aluminum stubs, and shadowed with gold. For visualization, a scanning electron microscope (Zeiss DSM 962) was used at 15 kV.

Stability of biofilm-negative variants. To test the stability of nonadhering *S. epidermidis* variants, single colonies were picked and incubated in fresh sterile TSB in a tissue culture flask (Greiner) at 37°C. After 24 h, the medium was replaced. This procedure was repeated until a biofilm of adhering bacteria became visible on the bottom of the flask (maximum of 15 days).

Pulsed-field gel electrophoresis. DNA isolation from *S. epidermidis* and pulsed-field gel electrophoresis were performed as described previously (15, 25).

Isolation of chromosomal DNA and restriction endonuclease cleavage. Bacterial strains were grown to the mid-log phase in Luria-Bertani broth (Difco) supplemented with 1% glycine at 37°C. Bacteria were harvested by centrifugation of 1.5 ml of the culture. The bacterial pellet was resuspended in 100 μl of buffer containing 25% sucrose, 10 mM Tris-HCl (pH 7.5), and 1 mg of lysostaphin (Sigma, Deisenhofen, Germany) per ml and incubated for 10 min at 37°C. After lysis of the bacterial cells, the chromosomal DNA was isolated by standard procedures (34).

After restriction endonuclease digestion, the DNA was analyzed by agarose gel electrophoresis (1% agarose in Tris-phosphate-EDTA).

Southern hybridization. Gels were blotted onto nylon membranes (Hybond-N^+; Amersham Life Science, Little Chalfont, England) by standard methods (34).

For hybridization, DNA of the plasmid pCN27 (21) was digested with *Eco*RI. The 2.6-kb *Eco*RI DNA fragment encoding *icaA* and the truncated *icaB* of the intercellular adhesin gene cluster (*ica*) of *S. epidermidis* RP62A was isolated by standard protocols (34). Probe labeling and hybridization were performed with the nonradioactive ECL direct nucleic acid labeling and detection system (Amersham Life Science). The hybridization buffer contained 1 M NaCl, and washing steps were performed at 55°C in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) containing 0.4% sodium dodecyl sulfate.

Northern (RNA) blot analysis of *ica* expression. For RNA extraction, the RNagents total RNA isolation system (Promega, Madison, Wis.) was used. Bacterial cells were grown in TSB to an OD_{600} of 2.0. Bacterial cells (10^{10}) were then pelleted, resuspended in 400 μl of diethylpyrocarbonate-treated water, and incubated with 100 μl of lysostaphin (1 mg/ml; Sigma) for 5 min at 37°C. The following RNA extraction and purification procedure was performed as recommended by the manufacturer.

Following DNase I digestion (fast-performance liquid chromatography-pure DNase I, RNase free; Pharmacia, Uppsala, Sweden), 30 μg of RNA was loaded onto 1% agarose gels containing 5% formaldehyde in 1× MOPS (morpholinepropanesulfonic acid) running buffer. Gel electrophoresis was performed at 5 V/cm for 3 h. The gel was blotted onto a nylon membrane (Hybond-N^+; Amersham Life Science) and hybridized with an ECL-labeled (Amersham Life Science) *ica*-specific DNA probe.

RESULTS

Biofilm formation and autoaggregation. The 88 *S. epidermidis* strains, i.e., 52 clinical isolates and 36 strains from body surfaces, were tested for their capacity to form biofilms on plastic material by a quantitative adherence assay (8). Thereby, a significant difference between the blood culture isolates and saprophytic strains could be observed. Among the former, 87% (45 of 52) formed biofilms on plastic, whereas only 11% (4 of 36) of the skin isolates produced a detectable biofilm on tissue culture plates.

The strains were screened for autoaggregation in TSB by light microscopy. In 79% (41 of 52) of the clinical isolates but only in 6% (2 of 36) of the skin and mucosal strains, large bacterial aggregates were detected. The data are summarized in Table 1.

Distribution of the intercellular adhesion-specific DNA sequence (*ica*). Recent data of Heilmann and coworkers (21) provided evidence that gene products of the *ica* locus play a crucial role in biofilm formation of *S. epidermidis*. To obtain information on the distribution of the *ica* gene cluster among *S. epidermidis* strains from several sources, the isolates were tested for the presence of the gene cluster by Southern hybridization of chromosomal DNA.

Following blotting onto nylon membranes, the chromosomal DNA was hybridized with an *ica*-specific gene probe. As demonstrated in Table 1, the gene was present in 85% (44 of 52) of the clinical *S. epidermidis* strains but only in 6% (2 of 36) of the saprophytic isolates. Thus, it appears that the presence of the *ica* gene is a common feature of *S. epidermidis* obtained from plastic material-associated infections but not of saprophytic isolates.

Correlation of colony morphology, autoaggregation, biofilm formation, and *ica* gene cluster presence. After incubation of staphylococcal strains on CRA plates, two major colony types could be distinguished by the color of their colonies. Nearly 87% (45 of 52) of the *S. epidermidis* blood culture isolates grew on CRA as black colonies, whereas only 6% (2 of 36) of the saprophytic strains formed black colonies.

Of the 88 *S. epidermidis* strains investigated, 49 strains, i.e., 45 from blood cultures and 4 from skin and mucosa, were found to be biofilm positive in the quantitative adherence assay. In 93% (46 of 49) of the biofilm-positive *S. epidermidis* strains, the *ica* gene cluster was present. In contrast, this DNA sequence was not detectable in biofilm-negative isolates.

Evaluation of all data on colony morphology, adherence, autoaggregation, and detection of the *ica* genes revealed a strong correlation between these features. Biofilm formation and autoaggregation were associated with the intercellular adhesion genes and the black colony type. This pattern was predominant in clinical isolates, whereas red colonies were more frequently observed in the nonadhering, adhesin-negative strains obtained from skin or mucosa.

Detection and characterization of phase variants. After plating a single, black colony of a blood culture strain onto CRA again, black colonies and a few red colonies emerged. The incidence of the emergence of the red colony type was approximately 10^{-5}. In the case of the saprophytic strains, no change of colony morphology could be observed, even after repeated passage of single colonies on CRA plates.

To decide whether the red colonies of clinical isolates represent contaminations or variants from the originally black parental strains, the variants were subcultivated and the restriction length polymorphism was tested by pulsed-field gel electrophoresis. These experiments revealed no detectable differences between the black and red colony forms in the genomic pattern (Fig. 1). However, the red variants were altered in their ability to form biofilms on polymer surfaces (Fig. 2B). To test the stability of the biofilm-negative variants, a single red colony of *S. epidermidis* 161 was picked for serial passage in TSB as described in Materials and Methods. This experiment revealed that the biofilm-negative phenotype of the variant is very stable. Only after ninefold serial passage of the nonadhering variant in TSB could the biofilm-producing phenotype be restored (Fig. 2B, strain 161/2).

Interestingly, the phenotypically different variants appeared to be identical on the DNA level as judged by pulsed-field gel electrophoresis. Therefore, we decided to analyze the basis of the phenotypic variation in more detail. To this end, the biofilm formation of *S. epidermidis* 161 and that of its biofilm-negative variant, strain 161/1, and its biofilm-positive revertant, strain 161/2, were investigated.

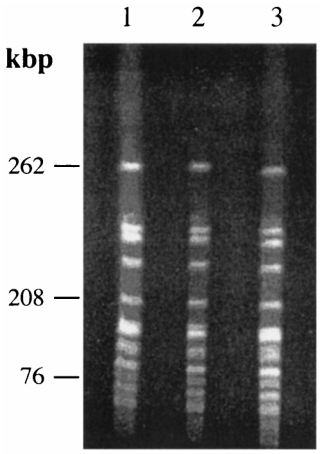

FIG. 1. Analysis of *Sma*I-digested genomic DNA from *S. epidermidis* 161 by pulsed-field gel electrophoresis. Lanes: 1, *S. epidermidis* 161; 2, biofilm-negative phase variant 161/1; 3, biofilm-forming revertant 161/2.

First, the initial adherence to polymer surfaces was studied. Initial adherence of staphylococci to polymers is mediated by a surface-associated, 60-kDa protein (20). The variants did not show any difference in their initial binding to a polymer surface (Fig. 2A). Consequently, it is most likely that an alteration of the second stage of biofilm formation is responsible for the biofilm-negative phenotype.

Second, the production of the extracellular slime substance (PIA) was investigated by immunofluorescence. PIA is a hexosamine-rich polysaccharide mediating the intercellular adherence of bacterial cells, and it was shown to be essentially involved in biofilm accumulation (28). It is synthesized by a polysaccharide synthetase which is presumably encoded by *icaA* (21). Immunofluorescence microscopy revealed clear evidence that the biofilm-negative variant does not produce PIA. In contrast, the biofilm-positive parent strain and the revertant were found to synthesize PIA (Fig. 2D). Since it had been shown that PIA production is necessary for autoaggregation and biofilm formation, the biofilm-negative phenotype of the variant can be explained by the absent PIA production. Moreover, immunofluorescence analysis showed that the PIA is mainly synthesized by those bacterial cells that are organized in large cell clusters, whereas bacteria that remain in suspension exhibit only a diminished adhesin production (Fig. 2C and D).

To get insight into the molecular mechanisms causing the biofilm-negative phenotype, Southern and Northern blot experiments were performed. Southern hybridization of chromosomal DNA with the *ica*-specific gene probe revealed that the genetic information is still present within the variants (Fig. 3). Therefore, the absence of PIA production cannot be caused by a complete deletion of the *ica* gene cluster.

The transcription of the *ica* operon of *S. epidermidis* 161 and that of its biofilm-negative variant, strain 161/1, were investigated by Northern blot analysis. Hybridization of total RNA with an *ica*-specific probe revealed that the transcription of the *ica* gene cluster is reduced in the PIA-negative phase variant (Fig. 4).

DISCUSSION

S. epidermidis is part of the epidermal microflora of healthy humans. However, during the last two decades, it has been demonstrated conclusively that this species and other coagulase-negative staphylococci are important pathogens causing

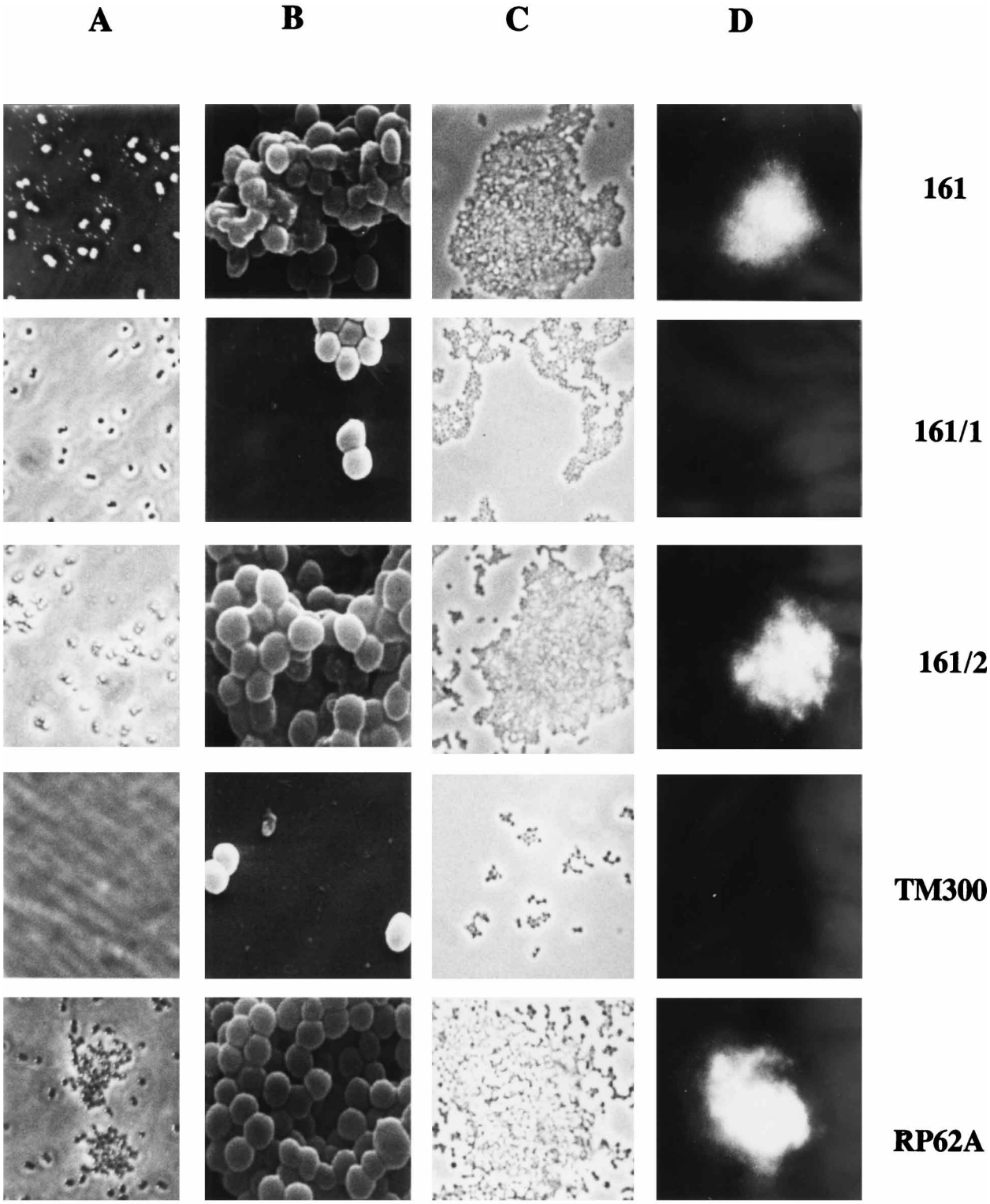

FIG. 2. Initial adherence, biofilm accumulation, autoaggregation, and PIA expression of *S. epidermidis* 161, its biofilm-negative variant, strain 161/1, its biofilm-positive revertant, strain 161/2, and *S. carnosus* TM300 and *S. epidermidis* RP62A as negative and positive controls, respectively. (A) Initial adherence to polystyrene petri dishes. Light microscopy of adhering bacteria after an incubation of bacterial cultures for 30 min at 37°C in TSB is shown. (B) Biofilm accumulation on polymer surfaces. Scanning electron micrographs of bacterial biofilms after an overnight incubation of bacterial cultures at 37°C in TSB as described in Materials and Methods are shown. (C) Detection of autoaggregates in liquid cultures after overnight incubation in TSB by phase-contrast microscopy. (D) Detection of PIA expression. Immunofluorescence micrographs of staphylococcal cells after incubation with the PIA-specific preadsorbed antiserum as described in Materials and Methods are shown. The immunofluorescence micrographs correspond to the phase-contrast pictures of panel C.

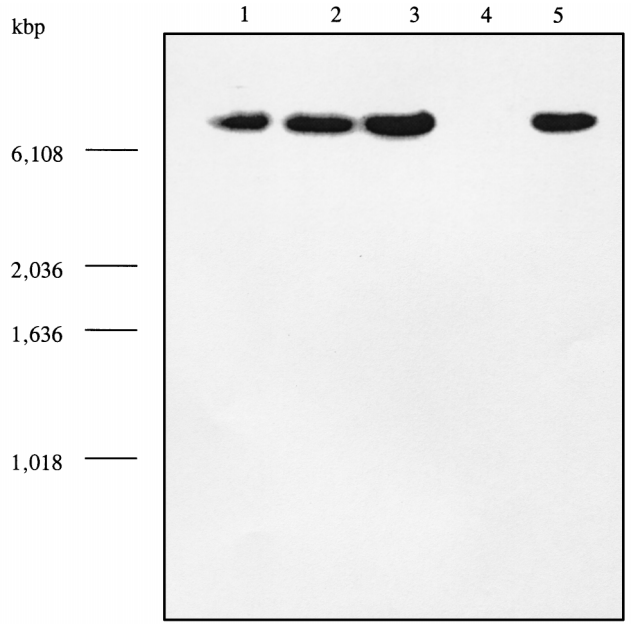

FIG. 3. Southern analysis of *S. epidermidis* 161. The chromosomal DNA was digested with *Bam*HI and hybridized with an *ica*-specific probe as described in Materials and Methods. Lanes: 1, biofilm-forming *S. epidermidis* 161; 2, nonadhering phase variant 161/1; 3, biofilm-forming revertant 161/2; 4, *S. carnosus* TM 300 (negative control); 5, *S. epidermidis* RP62A (positive control).

many nosocomial infections. Often, a correlation of infection with the use of medical devices can be observed. In the course of many studies, it became clear that the capacity of staphylococci to adhere to and grow on plastic surfaces is a crucial step in the pathogenesis of polymer-associated infections (7, 31, 32). Recently, several components that appear to be involved in adherence and slime production of staphylococci have been described (10, 20, 21, 27–29, 35, 39, 40). However, it remains unclear whether these factors are, indeed, virulence factors or simply common features of saprophytic staphylococci. It is important, therefore, to compare the properties of infectious agents and strains of the normal microflora.

In this study, we have compared *S. epidermidis* strains obtained from polymer-associated septicemic diseases with isolates obtained from skin and mucosa of healthy volunteers. The data clearly indicate genotypic and phenotypic differences among these groups of strains. First, the coding sequence of an intercellular adhesion gene cluster was found to be present in 85% (44 of 52) of *S. epidermidis* blood culture isolates compared to only 6% (2 of 36) of saprophytic strains. The two adhesin-positive strains that were found among the saprophytic isolates had been obtained from nasal swabs of two employees of an oncological ward, suggesting that this gene might be more prevalent in *S. epidermidis* strains isolated from hospital communities than in isolates obtained from normal healthy individuals.

Second, striking differences in terms of biofilm production and colony morphology on CRA between strains from different sources have been observed. In the quantitative adherence assay, 87% (45 of 52) of the blood culture isolates but only 11% (4 of 36) of the saprophytic strains adhered to plastic material. On CRA, the adhering strains formed black colonies whereas nonadhering strains developed red colony forms. Moreover, in liquid culture (TSB medium), the biofilm-producing isolates accumulated in bacterial aggregates that were detectable by light microscopy. Biofilm-negative strains, however, formed homogeneous suspensions in TSB medium. A strong correlation was found between biofilm production,

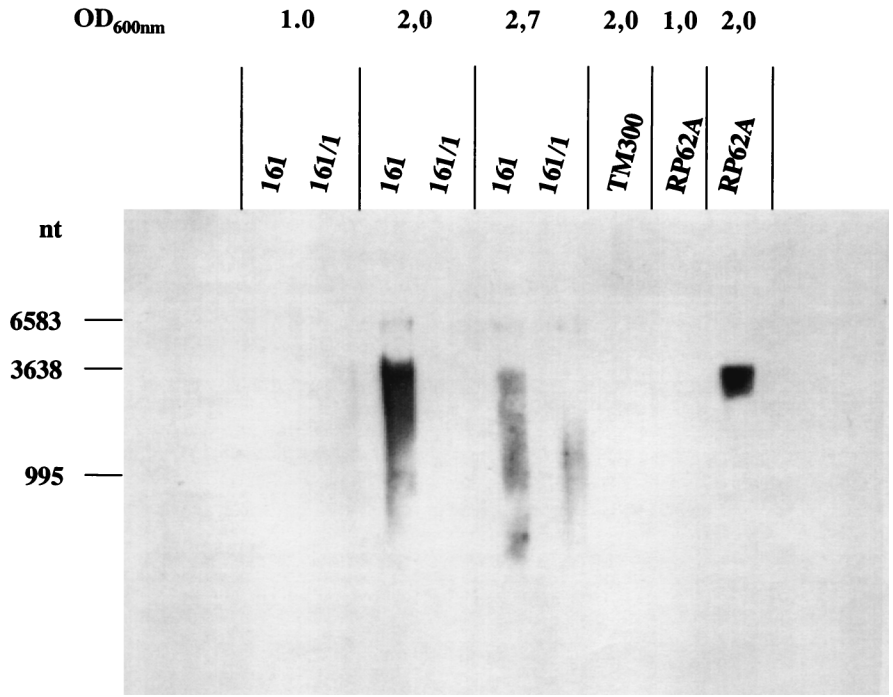

FIG. 4. Investigation of *ica* transcription by Northern blot analysis. RNAs of the biofilm-positive parent strain *S. epidermidis* 161 and its biofilm-negative variant, 161/1, were isolated and hybridized with the *ica*-specific probe at different ODs of the bacterial growth curve. *S. epidermidis* RP62A and *S. carnosus* TM 300 are positive and negative controls, respectively.

black colony type on CRA, autoaggregation, and the presence of the intercellular adhesion genes.

The *ica*-specific gene probe used for Southern hybridization originated from the slime-producing strain *S. epidermidis* RP 62A (21). The probe is specific for the *ica* operon coding for gene products required for the bacterial cell-to-cell contact and biofilm formation. The operon has been shown to contain at least three open reading frames. *icaA* encodes an enzyme that is anchored in the cytoplasmic membrane and probably acts as a polysaccharide synthetase mediating the synthesis of the PIA. A second open reading frame, *icaB*, which is located downstream of *icaA*, has been identified. It contains the information for an extracellular protein whose function is still unknown. The third open reading frame, *icaC*, encodes an integral membrane protein which is assumed to exert a receptor function for polysaccharide antigens. It is important to note that the simultaneous expression of these three staphylococcus gene products is required for PIA synthesis and the subsequent biofilm formation on polymer surfaces and autoaggregation in liquid cultures.

In biofilm-producing *S. epidermidis* strains, the expression of the intercellular adhesion genes (*ica*) was found to undergo a phase variation that correlates with a change of colony morphology on CRA and an altered biofilm formation. Biofilm-negative variants were unable to produce the PIA whose synthesis is mediated by the *ica* gene locus expression products. Genome analysis by pulsed-field gel electrophoresis and Southern hybridization revealed no detectable difference between the variants. Moreover, the biofilm-producing phenotype could be restored after repeated passages of a nonadhering single colony. These data lead us to conclude that the phenotypic change is not caused by a deletion of the adhesin gene. Northern blot analysis showed that the biofilm-negative phenotype of the variant is due to a reduced *ica* gene transcription. The molecular mechanisms underlying the regulation of *ica* transcription remain to be elucidated.

Nonadhering, red colony variants of *S. epidermidis* RP62A on CRA have also been described by Mempel et al. (30). These variants showed a remarkable increase of their methicillin susceptibility due to a lack of transcription of the methicillin resistance gene *mecA*. In the present study, 77% of the clinical *S. epidermidis* strains were resistant to oxacillin whereas only 8% of the saprophytic isolates were resistant. These data support the assumption that the *mecA* gene is more prevalent in the hospital environment. We also observed changes in the oxacillin susceptibilities of phenotypic variants (data not shown), supporting the suggestion of Christensen et al. (11) that heteroresistance to β-lactam antibiotics is a common feature of clinical staphylococcal isolates.

Our data on the distribution of the intercellular adhesion genes among clinical isolates suggest that the corresponding gene products could be considered a possible virulence factor in *S. epidermidis*. The intercellular adhesion gene expression was found to undergo a phase variation. Phenotypic changes of surface structures are common in many pathogenic bacteria. They have been observed in *Escherichia coli* adhesins (1, 4, 5, 16, 17, 26), in *Salmonella typhimurium* flagellum expression (36, 43), in antigenic and phase variation of *Neisseria* adhesins (18, 22, 24, 37, 38), and also in *Haemophilus influenzae* (41) and pneumococci (42). These studies have demonstrated that the heterogeneous expression of adhesins is important for the adaptation of bacteria to changing growth conditions and for the evasion of the host immune system. The phenotypic variation of the intercellular adhesin and the underlying genetic mechanisms are important scientific subjects. But clearly, more information is required to elucidate the role of the intercellular adhesin and its phenotypic variation in the pathogenesis of polymer-associated infections.

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