Med Microbiol Immunol (2002) 191: 101–106
DOI 10.1007/s00430-002-0124-3

ORIGINAL INVESTIGATION

Johannes K.-M. Knobloch · Matthias A. Horstkotte
Holger Rohde · Dietrich Mack

Evaluation of different detection methods of biofilm formation in *Staphylococcus aureus*

Received: 26 April 2002 / Published online: 29 June 2002

Abstract The *icaADBC* gene locus of *Staphylococcus aureus* and its polysaccharide intercellular adhesin (PIA/PNSG) were recently identified, but biofilm formation has rarely been detected in vitro. In this study we evaluated a tissue culture plate (TCP) assay and a tube test, as well as Congo red agar, using the two basic media trypticase soy broth (TSB) and brain heart infusion (BHI) broth with different sugar supplements for detection of biofilm formation in 128 *ica*-positive *S. aureus* isolates. Of the *S. aureus* strains, 57.1% displayed a biofilm-positive phenotype under optimized conditions in the TCP test. The tube test correlated well with the TCP test for strongly biofilm-producing strains, whereas weak producers were not safely discriminated from biofilm-negative strains. Screening on Congo red agar displayed a strong correlation with the TCP and the tube test for only 3.8%, and is therefore not recommended for investigation of biofilm formation in *S. aureus*.

Keywords *Staphylococcus aureus* · Biofilm · Congo red

Introduction

Staphylococci are the most important pathogens in foreign body-related infections. The predominant species isolated in these infections are *Staphylococcus epidermidis* and *Staphylococcus aureus* [42, 46]. In *S. epidermidis* the major pathogenetic factor is the ability to form a biofilm on polymeric surfaces [28]. This phenotype is sometimes described by the ambiguous term slime production [22]. For biofilm formation synthesis of an intercellular polysaccharide adhesin (PIA) is necessary mediating cell-to-cell adhesion [24]. PIA is synthesized by the gene products of the *icaADBC* locus [18, 20]. The significance of PIA expression and biofilm formation as a major pathogenetic factor of *S. epidermidis* was demonstrated in different animal models [43, 44, 45]. Recently homologous *icaADBC* genes were identified in *S. aureus* [9] and a closely PIA-related polysaccharide (PIA/PNSG) as well as biofilm/slime formation in vitro was observed in some of the *ica*-positive *S. aureus* strains [2, 3, 9, 16, 34]. Since elucidation of the mechanisms of biofilm formation of *S. aureus* may lead to new preventive measures, this phenotype is in the focus of present research [34]. Additionally, the differentiation of *S. aureus* with respect to its biofilm phenotype might help to elucidate the impact of *S. aureus* isolates in diagnosis of foreign body-related infections.

Different tests for the characterization of the biofilm phenotype have been established for *S. epidermidis*, which display a good correlation between a biofilm-positive phenotype and PIA expression [25]. The tests most often used are the qualitative tube test and the quantitative tissue culture plate (TCP) assay first established by Christensen et al. [7, 8] and modified by different investigators [14]. Additionally, screening on Congo red agar (CRA) was established by Freeman et al. [17], which has also been modified by different investigators [13, 19].

Investigation of the relation of *ica* genotype and biofilm formation of *S. aureus* using different methods led to contradictory results by different investigators. Cramton et al. [9] and Fowler et al. [16] characterized a total of 25 *S. aureus* isolates that were all *ica*-positive using PCR methods specific for the *S. aureus ica* locus. Only 4 of these isolates were biofilm-positive in a TCP assay, whereas the majority of the isolates were biofilm-negative. McKenney et al. [34] described 52 PIA/PNSG-positive strains out of 207 *S. aureus* isolates and 8 *ica*-positive strains by PCR specific for the *ica* gene locus

J.K.-M. Knobloch (✉) · M.A. Horstkotte
H. Rohde · D. Mack
Institut für Medizinische Mikrobiologie und Immunologie,
Universitätsklinikum Hamburg-Eppendorf,
Martinistr. 52, 20246 Hamburg, Germany
E-mail: knobloch@uke.uni-hamburg.de
Tel.: +49-40-428033147
Fax: +49-40-428034881

of *S. epidermidis*. Recently, Aricola et al. [2, 3] described detection of *ica* in only 25 out of 38 *S. aureus* strains by PCR specific for *S. epidermidis icaA* and *icaD*. In contrast to the results of the other investigators, all *icaADBC*-positive strains displayed a biofilm/slime-positive phenotype on CRA, whereas all *icaADBC*-negative strains were biofilm-negative on CRA. However, correlation of CRA and TCP assays for biofilm formation of *S. aureus* are unknown at present.

In this study we therefore evaluated the reliability of the indicated three methods for detection of biofilm formation in *S. aureus* and optimized the TCP assay for *S. aureus*.

Materials and methods

Bacteria and culture conditions

In total 128 non-copy *S. aureus* isolates were investigated. These included 82 blood culture isolates and 46 nasal *S. aureus* isolates of healthy medical students that were identified by standard microbiological techniques including colony morphology, clumping factor (Slidex Staph-Kit; bioMerieux, Lyon, France), and ID32Staph (bioMerieux). As controls the well-characterized *icaADBC*-positive, biofilm-producing *S. epidermidis* strains 1457, 9142, and 1057 and their isogenic biofilm-negative *icaA*-mutants 1457-M10, 1057-M10, and 9142-M10 were used [26, 30, 36]. Cells were grown in trypticase soy broth (TSB, Becton Dickinson, Cockeysville, Md.; Lot no. 1000G2DKUL), TSB supplemented with 1% glucose (TSB_{glc}) or 2% glucose and 2% sucrose ($TSB_{glc/suc}$), and brain heart infusion broth (BHI, Oxoid, Basingstoke, UK ; Lot no. 204999) supplemented with 3.6% sucrose (BHI_{suc}) or 2% glucose and 2% sucrose ($BHI_{glc/suc}$) at 37°C. For the CRA screening, TSB_{glc} (CRA_{TSB}) and BHI_{suc} (CRA_{BHI}) were supplemented with additional 0.08% Congo red (Merck, Darmstadt, Germany) and 1% agar (Becton Dickinson).

Genotypic and phenotypic characterization

For detection of *ica*, a PCR was established with oligonucleotides specific for *icaA* in *S. aureus* (SAicaA sense 5'-TGG CTG TAT TAA GCG AAG TC-3' and SaicaA antisense 5'-CCT CTG TCT GGG CTT GAC C-3'). Amplification of the resulting DNA fragments was performed using the DyNazyme DNA Polymerase Kit (Finzyme, Espoo, Finland) as described by the manufacturer. *S. aureus* strains were suspended in sterile water and boiled for 30 min. Of this suspension 5 µl was used as template in PCR reaction.

TCP assay was performed in the appropriate media in 96-well tissue culture plates (NunclonDelta; Nunc, Roskilde, Denmark) for 24 or 48 h as described [8, 29]. For the tube test, bacteria were grown in TSB for 5–6 h. Cultures were then diluted 1:100 in the appropriate media as indicated and incubated in glass tubes at 37°C without shaking for 48 h. Tubes were washed three times with deionized water and dried in an inverted position. Biofilms were stained with gentian violet (Merck). For the CRA screening, bacteria were plated on the respective agar and incubated at 37°C. Colony morphology and color were evaluated after 24, 48, and 72 h.

Results

The CRA screening as well as the tube test and the TCP test are well established for the detection of biofilm formation of *S. epidermidis* [14]. However, use of these tests for characterization of *S. aureus* biofilm formation has led to contradictory results by different investigators [2, 3, 9, 16, 34].

In our study in 128 *S. aureus* strains no *icaADBC*-negative strain was detected by a *icaA*-specific PCR, indicating that all *S. aureus* strains harbor this gene locus.

In the TCP assay, the standardized test for detection of *S. epidermidis* biofilm formation in our laboratory, which allows semiquantitative detection of biofilm formation, only 4 of 128 investigated *S. aureus* strains (3.1%) displayed a biofilm-positive phenotype in TSB after incubation for 24 or 48 h. Incubation for 48 h lead to better discrimination between biofilm-negative and biofilm-positive *S. aureus* strains. Therefore, a incubation time of 48 h was used for the further characterization of these strains (Table 1, Fig. 1).

Arciola et al. [2] reported that all *icaA*-positive *S. aureus* strains were strong slime producers on CRA composed from BHI supplemented with 3.6% sucrose (CRA_{BHI}). As biofilm formation of *S. aureus* varies depending on the environmental conditions [11, 38], we evaluated our strain collection in BHI_{suc} in the TCP test. Surprisingly 37 strains (28.9%) displayed a biofilm-positive phenotype in BHI_{suc} (Table 1, Fig. 1). Addition of 0.08% Congo red to the medium seemed to inhibit the primary attachment to the polystyrene surface and no biofilm formation could be detected (data not shown). Therefore, Congo red was omitted from all other media for TCP assays and tube tests.

In other studies CRA prepared from TSB supplemented with 1% glucose (CRA_{TSB}) was used [19]. We therefore also evaluated TSB_{glc} in the TCP assay. In this medium 49 strains (38.3%) were biofilm-positive (Table 1, Fig. 1). Interestingly, 14 of these strains were biofilm-negative in BHI_{suc}, whereas for 9 of the biofilm-

Table 1. Biofilm formation of 128 *Staphylococcus aureus* isolates (*TSB* trypticase soy broth, *BHI* brain heart infusion, TSB_{glc}/$TSB_{glc/suc}$ TSB supplemented with 1% glucose/2% glucose and 2% sucrose, BHI_{suc}/$BHI_{glc/suc}$ BHI supplemented with 3.6% sucrose/2% glucose and 2% sucrose)

Biofilm formation	TSB	TSB_{glc}	$TSB_{glc/suc}$	BHI_{suc}	$BHI_{glc/suc}$	Total
Negative	124	79	83	91	85	55
Positive	4	49	45	37	43	73
Medium dependent[a]	–	8	6	2	3	19
Basic medium dependent[b]	6			7		13

[a]Number of strains with biofilm-positive phenotypes in only this single medium
[b]Number of strains with biofilm-positive phenotypes in only one of the different basic media with supplementation by different sugars

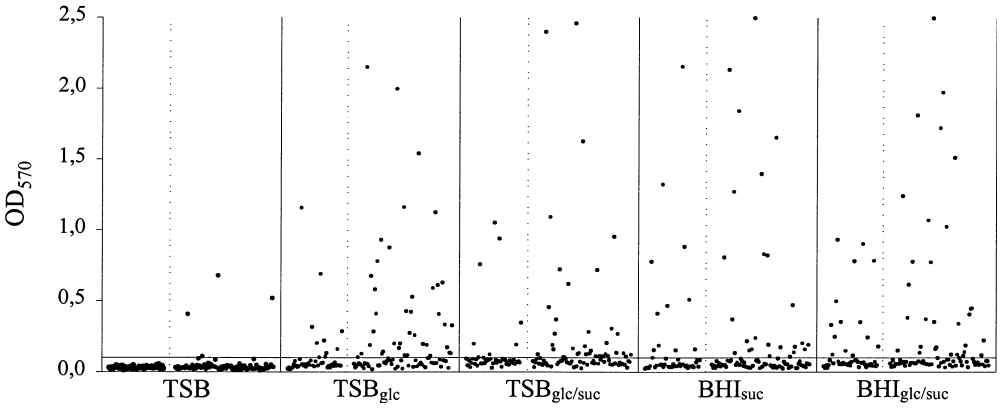

Fig. 1. Biofilm formation of *Staphylococcus aureus* in different media. Dots indicate the average OD_{570} of three independent TCP tests. For each medium the first 46 positions represent the nasal isolates separated by a dotted line from the following 82 positions representing the clinical isolates

positive strains in BHI_{suc} a biofilm-negative phenotype was observed in TSB_{glc} (Table 1). We therefore tested two additional media, TSB and BHI, both supplemented with 2% glucose and 2% sucrose ($TSB_{glc/suc}$, $BHI_{glc/suc}$), to investigate the influence of the basic medium and the respective sugar supplement (Table 1, Fig. 1). In $TSB_{glc/suc}$ 45 *S. aureus* strains (35.2%) were biofilm-positive. Six of these strains were only biofilm-positive in this medium, and 6 additional strains were only biofilm-positive in the media composed from TSB. In $BHI_{glc/suc}$ 43 strains (33.6%) displayed a biofilm-positive phenotype. Three of these strains were only biofilm-positive in this medium, and 7 additional strains formed biofilm only in media composed from BHI. In total, 73 (57.1%) of the investigated *S. aureus* isolates were biofilm-positive in at least one tested medium. The 4 strains with a biofilm-positive phenotype in unsupplemented TSB were positive in all media used.

The influence of the used media on biofilm formation differed between the clinical *S. aureus* isolates and the nasal isolates of healthy carriers. In sugar supplemented media composed from TSB, significantly more clinical isolates displayed a biofilm-positive phenotype (Table 2). In contrast, in media composed from BHI no statistical significant difference with respect to biofilm formation between clinical and nasal isolates was detected (Table 2). When evaluating the performance of all media used, significantly more clinical than nasal isolates were able to form biofilm in vitro (Table 2).

The tube test displayed a good correlation with the TCP assay for strongly biofilm-positive isolates in all tested media. However, for weakly biofilm-positive strains near the cutoff of the TCP assay a objective classification in biofilm-positive or biofilm-negative was difficult (data not shown).

For the evaluation of the CRA screening method, two different media CRA_{TSB} and CRA_{BHI} were used. Colony morphology was evaluated after 24, 48 and 72 h of incubation at 37°C. On CRA_{TSB} the majority of strains displayed black colonies on red agar without the typical dry crystalline morphology of the colonies known from biofilm-positive *S. epidermidis* strains, which were described as indeterminate by Freeman et al. [17]. Only five strains displayed the typical dry crystalline morphology. Four of these were the strains initially biofilm-positive in TSB and another strain strongly biofilm-positive strain in all supplemented media (examples of colony morphology are shown in Fig. 2). On CRA_{TSB}, only few (less than seven) strains displayed the red colony morphology typical for biofilm-negative *S. epidermidis* strains. Screening on CRA was controlled by three different biofilm-positive *S. epidermidis* wild-type strains and their corresponding biofilm-negative transposon mutants, which always exhibited the expected typical colony morphology. No significant changes in colony morphology was detected after 24, 48 and 72 h of incubation on CRA_{TSB}.

On CRA_{BHI}, all strains displayed red (ranging from pink to orange) colonies. A dry crystalline morphology of these red colonies was observed after 24 h for the five *S. aureus* strains with dry crystalline morphology on CRA_{TSB} as well as with the three *S. epidermidis* wild-type control strains (examples of colony morphology are shown in Fig. 2). This dry crystalline morphology extenuated after 48 and 72 h and the colonies lost their dry morphology beginning from the center of the colony. Few strains displayed colonies with an irregularly shaped contour without a dry crystalline morphology (Fig. 2). During incubation the agar changed its color from red to black after 24–48 h.

Table 2. Distribution of biofilm positive clinical and nasal *S. aureus* isolates and significance of differences

	TSB	TSB_{glc}	$TSB_{glc/suc}$	BHI_{suc}	$BHI_{glc/suc}$	Total
Nasal isolates	–	10	8	12	14	21
		21.7%	17.4%	26.1%	30.4%	45.7%
Clinical isolates	4	39	37	25	29	52
	4.8%	47.6%	45.1%	30.5%	35.4%	63.4%
Significance[a]	$P > 0.1$	$\underline{P < 0.01}$	$\underline{P < 0.01}$	$P > 0.5$	$P > 0.4$	$\underline{P < 0.01}$

[a]Significance was determined by Chi^2-test; significant values are *underlined*

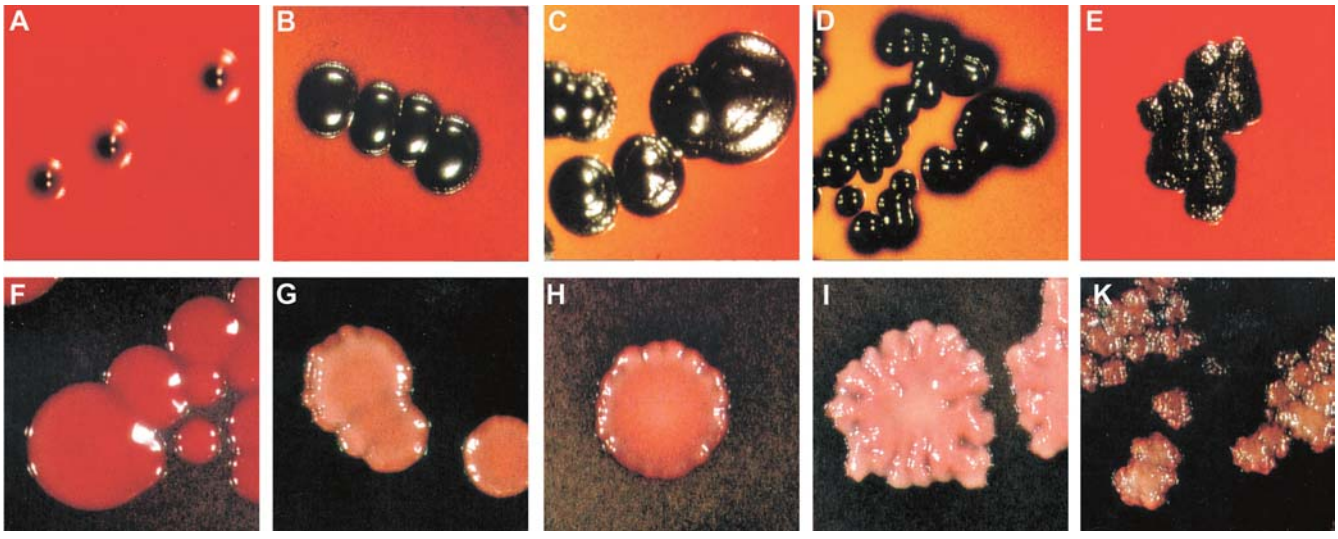

Fig. 2A–K. Colony morphologies of *S. aureus* on CRA. Different *S. aureus* isolates were cultivated on CRA_{TSB} (**A**–**E**) or CRA_{BHI} (**F**–**K**). Four biofilm-positive strains had the typical dry crystalline morphology seen in **E** and **K**. All other strains had morphologies consistent with biofilm-negative *S. epidermidis* strains (**A**, **F**) or intermediate morphologies (**B**–**D**, **G**–**I**) not correlating with their biofilm phenotype (*CRA* Congo red agar, *TSB* trypticase soy broth, *BHI* brain heart infusion)

With the exception of strains with dry crystalline morphology, no correlation of colony morphology on CRA and biofilm formation in the TCP assay was observed.

Discussion

The ability of *S. aureus* to form biofilm is a long known fact [4, 5, 6, 15, 31, 32, 33, 37], but the *icaADBC* gene locus and the polysaccharide PIA/PNSG mediating cell-to-cell adhesion was only described recently [9, 34]. With tests established for biofilm detection in *S. epidermidis*, contradictory results were obtained in respect of biofilm formation (0–100% of *icaADBC*-positive strains) by different investigators. Additionally, differential results were obtained in respect of the incidence of *icaADBC* in *S. aureus* by PCR and hybridization methods [2, 3, 9, 16, 34]. Therefore, we tested 128 well-characterized *S. aureus* strains for the presence of *ica*, and tried to optimize conditions for the detection of biofilm formation by *S. aureus* in vitro.

In our collection of 82 blood culture *S. aureus* isolates and 46 nasal isolates of healthy medical students, all strains harbored the *ica* locus as detected by a *S. aureus icaA*-specific PCR. These data correlate well with those reported by Fowler et al. [16] and Moore and Lindsay [35], who detected *ica* in all of 61 *S. aureus* isolates by PCR or hybridization techniques. The lower incidence of *icaADBC* reported by Arciola et al. [2, 3] could be due to the primers used for PCR amplification containing significant mismatches to the published *icaADBC* sequence of *S. aureus* [40]. In summary, 203 out of 212 (95.8%) *S. aureus* isolates with characterized *icaADBC* genotype were *ica*-positive and some of the additional strains might be falsely negative. These data suggest that *icaADBC* is present in virtually all *S. aureus* strains.

In the TCP assay with TSB used as the standard assay for detection of biofilm formation of *S. epidermidis* only 4 of 128 tested *S. aureus* strains (3.1%) displayed a biofilm-positive phenotype. This fact correlates favorably with the observations of other investigators in which only few or no biofilm producing *S. aureus* strains were detected [9, 16, 34]. Surprisingly, supplementation of TSB or BHI media with different sugars (TSB_{glc}, $TSB_{glc/suc}$, BHI_{suc}, $BHI_{glc/suc}$) increased biofilm formation significantly, and 57.1% of the investigated isolates formed biofilm in at least one of the used media. No significant difference was detected between clinical isolates and nasal isolates. It is noticeable that biofilm formation of 32 out of 73 strains (43.8%) was detectable in only one of the supplemented media or depends on one basic medium (TSB or BHI). These results indicate a strong dependence between biofilm formation in *S. aureus* and the environmental conditions of growth, which seems to be even more pronounced than in *S. epidermidis* [10, 23, 27, 29, 38, 39, 41, 47].

Interestingly, a significant difference of these effects was observed between clinical and nasal isolates in the TCP assay. Using TSB as the basic medium significantly more clinical isolates were able to form biofilm. In contrast, using media with BHI as the basic medium the number of biofilm-positive strains increased for the nasal isolates and decreased for the clinical isolates, leading to a statistically non-significant difference of these populations. These results suppose that different regulatory mechanisms could be active in expression of biofilm in infectious and commensal *S. aureus*, as is also indicated by the observation that infectious and commensal *S. aureus* clones represent different but overlapping clonal lineages [12].

The tube test correlates well with the TCP test for strongly biofilm-producing *S. aureus* strains. In contrast, weakly biofilm-producing strains were separated from biofilm-negative strains only with difficulty due to the variability and subjectivity of this assay.

For both CRA used in this study, a positive correlation of colony morphology and biofilm formation was observed for only 5 out of 128 strains (3.9%). These strains displayed typical dry and crystalline colonies with a black color on red agar on CRA_{TSB} and a red color on black agar on CRA_{BHI}. For all other strains many different colony morphologies without any correlation to biofilm formation in the TCP or tube test was observed. We expect that the different colony morphologies were induced by interaction of Congo red with different capsular polysaccharides produced by the majority of *S. aureus* strains [1, 21] independent of the production of PIA/PNSG.

For further investigations the use of the TCP assay with both TSB_{glc} and $BHI_{glc/suc}$ is recommended, which detected 65 out of 73 strains (89%) with the potential of biofilm expression in vitro. It is possible that due to differences in the composition of media additional single strains could be induced to form biofilm, as it was the case with the 8 strains that were not detected in the two recommended media, but the efforts to detect these strains may exceed the advantage in diagnosis. The tube test may be an easy screening assay for strongly biofilm-producing strains, but we cannot recommend this test as a general screening assay for biofilm formation due to the difficult classification of weakly biofilm-positive *S. aureus* strains. The CRA method cannot be recommended at all as a method for detection of biofilm formation in *S. aureus* because there is almost no correlation between colony morphology and biofilm-positive or biofilm-negative phenotype in the TCP or tube test. It could be a useful method for screening for phase variants or mutants with reduced biofilm formation of strongly biofilm-positive *S. aureus* strains, which display the typical dry crystalline morphology on this agar.

Acknowledgements We thank Rainer Laufs for his continuous support. This work is supported by a grant of the Deutsche Forschungsgemeinschaft, given to D.M..

References

1. Arbeit RD, Karakawa WW, Vann WF, Robbins JB (1984) Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. Diagn Microbiol Infect Dis 2:85–91
2. Arciola CR, Baldassarri L, Montanaro L (2001) Presence of *icaA* and *icaD* genes and slime production in a collection of staphylococcal strains from catheter-associated infections. J Clin Microbiol 39:2151–2156
3. Arciola CR, Collamati S, Donati E, Montanaro L (2001) A rapid PCR method for the detection of slime-producing strains of *Staphylococcus epidermidis* and *S. aureus* in periprosthesis infections. Diagn Mol Pathol 10:130–137
4. Baselga R, Albizu I, De La CM, Del Cacho E, Barberan M, Amorena B (1993) Phase variation of slime production in *Staphylococcus aureus*: implications in colonization and virulence. Infect Immun 61:4857–4862
5. Brock JH, Reiter B (1976) Chemical and biological properties of extracellular slime produced by *Staphylococcus aureus* grown in high-carbohydrate, high-salt medium. Infect Immun 13:653–660
6. Caputy GG, Costerton JW (1982) Morphological examination of the glycocalyces of *Staphylococcus aureus* strains Wiley and Smith. Infect Immun 36:759–767
7. Christensen GD, Simpson WA, Bisno AL, Beachey EH (1982) Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun 37:318–326
8. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, Beachey EH (1985) Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 22:996–1006
9. Cramton SE, Gerke C, Schnell NF, Nichols WW, Götz F (1999) The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect Immun 67:5427–5433
10. Cramton SE, Gerke C, Götz F (2001) In vitro methods to study staphylococcal biofilm formation. Methods Enzymol 336:239–255
11. Cramton SE, Ulrich M, Götz F, Döring G (2001) Anaerobic conditions induce expression of polysaccharide intercellular adhesin in *Staphylococcus aureus* and *Staphylococcus epidermidis*. Infect Immun 69:4079–4085
12. Day NP, Moore CE, Enright MC, Berendt AR, Smith JM, Murphy MF, Peacock SJ, Spratt BG, Feil EJ (2001) A link between virulence and ecological abundance in natural populations of *Staphylococcus aureus*. Science 292:114–116
13. Deighton MA, Capstick J, Borland R (1992) A study of phenotypic variation of *Staphylococcus epidermidis* using Congo red agar. Epidemiol Infect 109:423–432
14. Deighton MA, Capstick J, Domalewski E, Nguyen T van (2001) Methods for studying biofilms produced by *Staphylococcus epidermidis*. Methods Enzymol 336:177–195
15. Ekstedt RD, Bernhard JM (1973) Preparation and characterization of a slime layer material produced by *Staphylococcus aureus*. Proc Soc Exp Biol Med 142:86–91
16. Fowler VG, Fey PD, Reller LB, Chamis AL, Corey GR, Rupp ME (2001) The intercellular adhesin locus *ica* is present in clinical isolates of *Staphylococcus aureus* from bacteremic patients with infected and uninfected prosthetic joints. Med Microbiol Immunol (Berl) 189:127–131
17. Freeman DJ, Falkiner FR, Keane CT (1989) New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol 42:872–874
18. Gerke C, Kraft A, Süssmuth R, Schweitzer O, Götz F (1998) Characterization of the *N*-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. J Biol Chem 273:18586–18593
19. Heilmann C, Götz F (1998) Further characterization of *Staphylococcus epidermidis* transposon mutants deficient in primary attachment or intercellular adhesion. Zentralbl Bakteriol 287:69–83
20. Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Götz F (1996) Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol Microbiol 20:1083–1091
21. Hochkeppel HK, Braun DG, Vischer W, Imm A, Sutter S, Staeubli U, Guggenheim R, Kaplan EL, Boutonnier A, Fournier JM (1987) Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide types 5 and 8. J Clin Microbiol 25:526–530
22. Hussain M, Wilcox MH, White PJ (1993) The slime of coagulase-negative staphylococci: biochemistry and relation to adherence. FEMS Microbiol Rev 10:191–207
23. Knobloch JKM, Bartscht K, Sabottke A, Rohde H, Feucht HH, Mack D (2001) Biofilm formation by *Staphylococcus*

epidermidis depends on functional RsbU, an activator of the *sigB* operon: differential activation mechanisms due to ethanol and salt stress. J Bacteriol 183:2624–2633
24. Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H, Laufs R (1996) The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. J Bacteriol 178:175–183
25. Mack D, Haeder M, Siemssen N, Laufs R (1996) Association of biofilm production of coagulase-negative staphylococci with expression of a specific polysaccharide intercellular adhesin. J Infect Dis 174:881–884
26. Mack D, Riedewald J, Rohde H, Magnus T, Feucht HH, Elsner HA, Laufs R, Rupp ME (1999) Essential functional role of the polysaccharide intercellular adhesin of *Staphylococcus epidermidis* in hemagglutination. Infect Immun 67:1004–1008
27. Mack D, Rohde H, Dobinsky S, Riedewald J, Nedelmann M, Knobloch JKM, Elsner H-A, Feucht HH (2000) Identification of three essential regulatory gene loci governing expression of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin and biofilm formation. Infect Immun 68:3799–3807
28. Mack D, Bartscht K, Dobinsky S, Horstkotte MA, Kiel K, Knobloch JKM, Schäfer P (2000) Staphylococcal factors involved in adhesion and biofilm formation on biomaterials. In: An YH, Friedman RJ (eds) Handbook for studying bacterial adhesion: principles, methods, and applications, 1st edn. Humana Press, Totowa, pp 307–330
29. Mack D, Bartscht K, Fischer C, Rohde H, Grahl C de, Dobinsky S, Horstkotte MA, Kiel K, Knobloch JKM (2001) Genetic and biochemical analysis of *Staphylococcus epidermidis* biofilm accumulation. Methods Enzymol 336:215–239
30. Mack D, Sabottke A, Dobinsky S, Rohde H, Horstkotte MA, Knobloch JKM (2002) Differential expression of methicillin resistance by different biofilm-negative *Staphylococcus epidermidis* transposon mutant classes. Antimicrob Agents Chemother 46:178–183
31. Marrie TJ, Costerton JW (1984) Scanning and transmission electron microscopy of in situ bacterial colonization of intravenous and intraarterial catheters. J Clin Microbiol 19:687–693
32. Marrie TJ, Nelligan J, Costerton JW (1982) A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation 66:1339–1341
33. Marrie TJ, Noble MA, Costerton JW (1983) Examination of the morphology of bacteria adhering to peritoneal dialysis catheters by scanning and transmission electron microscopy. J Clin Microbiol 18:1388–1398
34. McKenney D, Pouliot KL, Wang Y, Murthy V, Ulrich M, Doring G, Lee JC, Goldmann DA, Pier GB (1999) Broadly protective vaccine for *Staphylococcus aureus* based on an in vivo-expressed antigen. Science 284:1523–1527
35. Moore PC, Lindsay JA (2001) Genetic variation among hospital isolates of methicillin-sensitive *Staphylococcus aureus*: evidence for horizontal transfer of virulence genes. J Clin Microbiol 39:2760–2767
36. Nedelmann M, Sabottke A, Laufs R, Mack D (1998) Generalized transduction for genetic linkage analysis and transfer of transposon insertions in different *Staphylococcus epidermidis* strains. Zentralbl Bakteriol 287:85–92
37. Raad II, Bodey GP (1992) Infectious complications of indwelling vascular catheters. Clin Infect Dis 15:197–208
38. Rachid S, Ohlsen K, Wallner U, Hacker J, Hecker M, Ziebuhr W (2000) Alternative transcription factor sigma(B) is involved in regulation of biofilm expression in a *Staphylococcus aureus* mucosal isolate. J Bacteriol 182:6824–6826
39. Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W (2000) Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. Antimicrob Agents Chemother 44:3357–3363
40. Rohde H, Knobloch JKM, Horstkotte MA, Mack D (2001) Correlation of *Staphylococcus aureus icaADBC* genotype and biofilm expression phenotype. J Clin Microbiol 39:4595–4596
41. Rohde H, Knobloch JKM, Horstkotte MA, Mack D (2001) Correlation of biofilm expression types of *Staphylococcus epidermidis* with polysaccharide intercellular adhesin synthesis: evidence for involvement of *icaADBC* genotype-independent factors. Med Microbiol Immunol (Berl) 190:105–112
42. Rupp ME, Archer GL (1994) Coagulase-negative staphylococci: pathogens associated with medical progress. Clin Infect Dis 19:231–243
43. Rupp ME, Ulphani JS, Fey PD, Bartscht K, Mack D (1999) Characterization of the importance of polysaccharide intercellular adhesin/hemagglutinin of *Staphylococcus epidermidis* in the pathogenesis of biomaterial-based infection in a mouse foreign body infection model. Infect Immun 67:2627–2632
44. Rupp ME, Ulphani JS, Fey PD, Mack D (1999) Characterization of *Staphylococcus epidermidis* polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. Infect Immun 67:2656–2659
45. Rupp ME, Fey PD, Heilmann C, Götz F (2001) Characterization of the importance of *Staphylococcus epidermidis* autolysin and polysaccharide intercellular adhesin in the pathogenesis of intravascular catheter-associated infection in a rat model. J Infect Dis 183:1038–1042
46. Thylefors JD, Harbarth S, Pittet D (1998) Increasing bacteremia due to coagulase-negative staphylococci: fiction or reality? Infect Control Hosp Epidemiol 19:581–589
47. Vuong C, Saenz HL, Götz F, Otto M (2000) Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis 182:1688–1693