III. Materials

1. Bacterial strains

The *E. coli* strains used in this thesis, are listed in table 1.

Table 1: *E. coli* strains used in this study

E. coli K-12-strains	Characteristics	Source or reference
DH5α	F^-, *endA*1, *hsdR*17 (r_k^-, m_k^-), *supE*44, *thi*-1, *recA*1 *GyrA*96, *relA*1, λ$^-$, Δ(argF-lac)U169, Φ80*dlacZ*ΔM15	[118]
MC4100	F^-, *araD*139, Δ(*argF-lac*)U169, *rspL*150, *relA*1, *deoC*1, *ptsF*25, *rsbR*, *flbB*5301	[119]
XL1-blue	*SupE*44, *hsdR*17, *recA*1, *endA*1, *gyrA*46, *thi*, *relA*1, *lac*$^-$, F^- (*proAB*$^+$, *lacI*^{q}, *lacZ*Δ M15, *Tn*10 (tet^r))	[120]

All *Staphylococcus*- and *Bacillus* strains used in this thesis, are listed in table 2.

Table 2: *Staphylococcus*- and *Bacillus* strains used in this study

Staphylococcus strains	Characteristics	Source or reference
S. epidermidis 220	Blood culture isolate, *ica*- positive, biofilm positive; pen^s; Gm^s; Ox^s; Em^r; Cm^s; Tc^s; Cc^s; Va^s; Te^s; Q/D^s; origin of template DNA for PCR amplification of the *ica* promotor segment (P_{ica}); host for chromosomal integration of plasmid pSK2	This study
S. epidermidis 220-1	Derivative of *S. epidermidis* 220 with chromosomal P_{ica}*:::lacZ* fusion, Cm^r, Em^r	This study
S. epidermidis RP62A	Blood culture isolate, *ica*-positive	ATCC 35984
S. epidermidis 215	Blood culture isolate, *ica*-positive	This study
S. epidermidis 561	Clinical strain, *ica*- positive, biofilm negative (*in vitro*); isolated from catheter-related urinary tract infection	This study

S. epidermidis 567	Clinical strain, *ica*-positive; isolated from catheter-related urinary tract infection case	This study
S. epidermidis 567-1	Derivative of *S. epidermidis* 567, *agr* mutant, Em^r	This study
S. aureus RN4220	NCTC 8325-4-RNA (restriction mutant) efficient acceptor for *E. coli* DNA	[121]
S. aureus MA12	MSSA, biofilm positive	[122]
S. aureus MA12.2	*S. aureus* MA12, *sigB* mutant, Em^r	This study
S. aureus MA12.2-1	*S. aureus* MA12.2, carrying a pSK9 plasmid	This study
S. epidermidis 195	Skin isolates, *ica* negative	[73]
S. epidermidis 195-1	*S. epidermidis* 195 carrying a pSK2 plasmid	This study
Bacillus subtilis-168	*TrpC2*	[123]
Bacillus subtilis-168-1	*Bacillus subtilis*-168 carrying a pSK2	This study

2. Plasmids and gene probes

All the plasmids used in this work, are listed in table 3.

Table 3: Plasmids and gene probes

Plasmids	Vector/characteristics	Source or reference
pUC18/19	Amp^r, *lacz*α, ori ColE1	[124]
pSK1	pUC18, carrying the *ica*-promotor segment of *S. epidermidis* 220; Amp^r	This study
pKO10	Derivative of pBT1 shuttle vector [125], Amp^r, Cm^r, ori ColE1, ori (ts), carrying a *hla::lacZ*-fusion	[126]
pSK2	pBT1 shuttle vector Amp^r, Cm^r, ori ColE1, ori (ts), carrying a P_{ica}::-*lacZ*-fusion	This study
pGEM®-T Easy	pGEM®-T and pGEM®-T Easy vector system for direct PCR cloning, Amp^r	Promega
pSK3	pGEM®-T Easy containig a 2.4 kb fragment of the *agr* from *S. epidermidis* 220	This study

pSK4	pSK3, *agr::ermB*	This study
pSK5	pBT1 shuttle vector, Amp^r, Cm^r, ori ColE1, ori (ts), carrying a *agr::ermB* fragment from pSK4	This study
pSK6	pUC18, carrying the *sigB* operon of *S. aureus*	This study
pSK7	pBT1, carrying the *sigB* operon of *S. aureus*	This study
pSK8	pBT1, carrying a *sigB::ermB* fragment	This study
pHPS9	Shuttle vector, Cm^r, Em^r, carrying a *cat-86::lacZ*α gene fusion	[127]
pSK9	pHPS9 shuttle vector carrying the *sigB* operon of *S. aureus*	This study
pEC1	pUC18, carrying the *ermB* gene.	[125]

3. Oligonucleotides

The oligonucleotides used in this research are listed in table 4 and 5.

Table 4: Oligonucleotides and their usage

Nucleotides	Sequence (5´- 3´)	Usage
Pica-1	TGT TTG ATT TCT GAA TTC AGT GCT TCT GGA GC	*Pica*-5`-Primer
Pica-2	TTC AGG ATA TTC TAG AGA TAA AAC ACT AG	*Pica*-3`-Primer
LacZ-1	GTT ACG TTG GTC TAG ATG GGC GCA TCG	*LacZ*-3`-Primer
Agr-1	GAG GAT CCG AGT GAC AAG TAG GAT ACT	*Agr*-5`-Primer
Agr-2	GAG GAA TTC CTC GTG CCA ATG TTA CGT	*Agr*-3`-Primer
Agr-3	GAG TAT AGT GTC ACT ACA CTA AC	
Agr-4	AGA GAC TCA CGG CTT GAT AAC	
SigB-1	CGG GAT CCG GTG TGA CAA TCA GTA TGA C	*SigB*-5`-Primer
SigB-2	CGG AAT TCG CGA CAT TTA TGT GGA	*SigB*-3`-Primer

	TAC AC	
Asp23-1	GGA GAA TCT ATT ATG ACT GT	*Asp23*-5`-Primer
Asp23-2	GTC GGC ACT AAA ATG GTG TG	*Asp23*-3`-Primer

Table 5: Fluorescence labelled oligonucleotides

Agr-eryth-1	GCG TAC CGT GTG CAT GTC	*ErmB*-5`-Primer
Agr-eryth-2	GGG ATG GCT CAA CAA CTC	*Agr*-3`-Primer
ica-lacZ-1	ATC ATC AAG TGT ATG ACC GT	*Ica*-5`-Primer
ica-lacZ-2	TTA ATG AAT CGG CCA ACG C	*LacZ*-3`-Primer
M13 Universal	TGT AAA ACG ACG GCC AGT	Cloning site-5`-Primer
M13 Reverse	CAG GAA ACA GCT ATG ACC	Cloning site-3`-Primer

4. **Chemicals**

Chemicals were obtained from the following companies:
Boehringer, Mannheim, Germany; Difco, Augsburg, Germany; Fluka, Deisenhofen, Germany; Gibco, Eggenstein, Germany; Merck, Darmstadt, Germany; Oxoid, Wesel, Germany, Roth, Karsruhe, Germany; Serva, Heidelberg, Germany; and Sigma, Deisenhofen, Germany.
The used enzymes were purchased from Boehringer; Eurogentec, Searing, Belgium; Gibco; Serva; Pharmacia, München, Germany; and Promega, Heidelberg, Germany.
α- ^{32}P- dATP was purchased from Amersham, Braunschweig, Germany.

The following Kits were used:

- ECL, Amersham,
- Galacto-Light Plus™, Perkin Elmer, Weiterstadt, Germany,
- Gene Clean, Dianova, Hamburg, Germany,
- Random primed DNA labelling, Boehringer,
- RNeasy™, Qiagen, Hilden, Germany,
- FastRNA Blue-kit, Bio101, Dianova, Hamburg, Germany

- Sure Clone® Ligation, Pharmacia,
- Thermo Sequenase fluorescence-labelled primer cycle sequencing kit, Amersham Life Science

5. Equipments

All the equipments needed for this work are:

- Autoclave Fedegari-FOM/B50
- Camera Nikon F301
- Cell disruptor apparatus (Savant Instrument), Dianova
- Centrifuge Heraeus Biofuge 13R
  Heraeus Megafuge 1.0R
  Eppendorf 5415 C
  Beckman J 2-21
- Clean bench Nunc Inter Med
- Computer Pentium 230 MHZ
- Computer programs Microsoft Office 4.2
- Electronic balance Chyo MP-3000
  Chyo JL-180
- Electrophoresis chamber BioRad
- Electroporator BioRad, Gene Pulser transfection
- Films Hyperfilm ECL
  Fuji X-ray film RX
- Freezer (80) Revco
- Gene linker BioRad
- Hot Plate Eppendorf Thermostat 5320
- Hybridization oven HybAid
- Ice machine Scotman AF-20
- Incubator Mammert Tv40b
  Heraeus B5050E
- Luminometer Lumat LB9501, Berthold
- Magnetic stirrer GLW
- Micro pipettes Gilson, Eppendorf

- Microplate reader BioRad
- Microtiter plates 96 well Greiner, FALCON
- Microwave oven Moulinex
- Nylon membrane Biodyne B, Pall
- Oil vacuum pump Univac Uniequipe
- PCR- Thermocycler Techne Progene, eppendorf
- pH-Meter WTW pH 523
- Photometer Pharmacia
- Platform Shaker STR6 Scientific
- Power supply BioRad 200mA, 500V
- Printer HP Laserjet 6MP
- Rotation mixer eppendorf mixer 5432
- Scanner HP ScanJet Iicx
- Sequence analyzer MWG-Biotech LI-GOR-4000
- Shaker GLW
  GFL Wasserbad
  Innova TM 4300
- Speedvac-concentrator UNIVAPO 150H Uniequipe
- Sterile filter Schleicher & Schuell 0.22µm
- Vacuum-blotter Pharmacia
- Vacuum oven Heraeus
- Video printer Mitsubishi, Hitachi, Cybertech Cb 1
  Biometra, Bio-Rad
- Vortexer GLW
- Water bath GFL 1083, Köettermann

6. Media and supplements

- Distilled water was used for all media.
- All media were autoclaved for 20 minutes.

- For agar plates, 12 g bacteriological agar (gibco BRL) per one liter of medium was added before autoclaving.

Luria-bertani (LB) medium:

Bactotryptone (or casein-hydrolysat-peptone) (10 g/l), yeast extract (5 g/l), NaCl (10 g/l), H_2O.

Brain Heart Infusion Broth (BHI) (Difco):

37 g per one liter distilled H_2O.

Müller-Hinton Broth (Oxoid):

12 g per one liter distilled H_2O.

Tryoticase Soybean Broth (TSB) (Difco):

30 g per one liter distilled H_2O.

DM3 agar:

Prepared by a mixture of seven components.

Component 1 (200 ml)

Bacto agar	5%

Component 2 (500 ml)

Na-succinat	1M

pH adjusted to 7.3 with Succinic acid

Component 3 (150 ml)

Casein-hydrolysat-pepton	3.3%
Yeast extract	3.3%

Component 4 (100 ml)

K_2HPO_4	3.5%
KH_2PO_4	3.5%

Component 5 (20 ml)

$MgCl_2 . 6H_2O$	1M

Component 6 (10 ml)

Glucose	50%

Component 7 (10 ml)

BSA	5%

All the components autoclaved separately, component 7 sterilised by filtration.

SMMP$_{75}$: Consists of:

7.5 parts 2x SMM
2.0 Parts 4x PAB
0.5 Part BSA-solution (5%)

A) 2x **SMM**

Sucrose	1M
Maleic acid	40 mM
$MgCl_2$. $6H_2O$	40mM

pH was adjusted to 6.8 with NaOH and later autoclaved at 121°C for 7 minutes.

B) 4x **PAB** (70 g/l Pennassy broth)
Difco antibiotic medium 3, autoclaved at 121°C for 15 minutes.

C) **BSA-solution** (5%)

BSA	5 g
2x SMM	100 ml

pH adjusted to 7.5 with NaOH, and sterilised by filtration through 0.22 micron filter.

LB-X-Gal-Plates:

After cooling the LB agar to 45°C, 3 ml of 2% X-Gal solution (5-bromo-4-chloro-3-indolyl-β-galactoside in dimethylformamide) and 500 µl IPTG (100 mM) as well as the necessary antibiotics were added per one liter medium.

Chemically defined medium (CDM) prepared for biofilm production in *S. epidermidis*

Consists of five groups of chemicals, listed in table 6.

Table 6: Chemically defined medium

Group (1)

Materials	Weight/liter
$FeSO_4$. $7H_2O$	5.0 mg
K_2HPO_4	200 mg
KH_2PO_4	200 mg
$MgSO_4$. $7H_2O$	5.0 mg
$MnSO_4$	5.0 mg

Group (2)

Materials	Weight/liter
L-alanine	100 mg
L-argenine	100 mg
L-aspartic acid	100 mg
L-cystine	50 mg
L-glutamic acid	100 mg
L-glycine	100 mg
L- histidine	100 mg
L-isoleucine	100 mg
L-leucine	100 mg
L-lysine	100 mg
L-methionine	100 mg
L-phenylalanine	100 mg
Proline	100 mg
Hydroxy-L-proline	100 mg
L-serine	100 mg
L-therionine	200 mg
L-tryptophan	100 mg
L-thyrosine	100 mg
L-valine	100 mg

Group (3)

Materials	Weight/liter
P-aminobenzoic acid	0.2 mg
Biotin	0.2 mg
Niacinamide	1.0 mg
β-nicotinamide adenine dinucleotide	2.5 mg
Pyridoxamine	1.0 mg
Riboflavin	2.0 mg

Group (4)

Materials	Weight/liter
Adenine	20 mg
Guanine hydrochloride	20 mg
Uracil	20 mg

Group (5)

Materials	Weight/liter
Glucose	5000 mg
$CaCl_2$. $6H_2O$	10 mg
Na_2HPO_4	300 mg
$NaHCO_3$	500 mg

The above groups were prepared separately and mixed together after sterilization (group 1 and 5 were autoclaved, group 2, 3, and 4 were filter sterilized), pH was adjusted to 7.0.

7. Antibiotics

- Stock solutions of water soluble antibiotics were prepared in distilled H_2O and sterilized by filtration through a 0.22-micron filter.
- Antibiotics soluble in ethanol were dissolved in 96% ethanol and were not sterilized.
- All antibiotics were added to the medium after cooling it to 45°C.

The antibiotics used in this thesis, are listed in table 7.

Table 7: List of the antibiotics used in this study

Antibiotics	Source	Stock solution
Ampicillin	Sigma, Deisenhofen, Germany	100 mg/ml in H_2O
Chloramphenicol	Serva, Heidelberg, Germany	10 mg/ml in EtOH
Clindamycin	Sigma	10 mg/ml in H_2O
Erythromycin	Sigma	10 mg/ml in H_2O
Fusidic acid	Sigma	10 mg/ml in H_2O
Gentamicin	Sigma	10 mg/ml in H_2O
Ofloxacin	Roussel Uclaf	10 mg/ml in acetic acid
Oxacillin	Sigma	10 mg/ml in H_2O
Penicillin G	Sigma	10 mg/ml in H_2O
Quinupristin/Dulfopristin	Rhone-poulenc	10 mg/ml in H_2O
Dulfopristin (RP 54476)	Rhone-poulenc	10 mg/ml in H_2O
Quinipristin (RP 57669)	Rhone-poulenc	10 mg/ml in H_2O
Teicoplanin	Roussel Uclaf	10 mg/ml in H_2O
Tetracycline	Sigma	10 mg/ml in H_2O
Vancomycin	Sigma	10 mg/ml in H_2O
Viriginamycin	Sigma	10 mg/ml in H_2O

8. Buffer and solutions

8.1 Electrophoresis running buffers

10x TPE-buffer
108 g Tris
15 ml 85% phosphoric acid
40 ml 0.5 M EDTA pH 8.0
add 1 l H_2O

50x TAE-buffer
242 g Tris
57.1 ml glacial acetic acid
100 ml 0.5 m EDTA pH 8.0
Add 1 l H_2O

6x Loading buffer
0.25% bromophenol blue
0.25% xylen-cyanol
15% Ficoll type 400 in H_2O