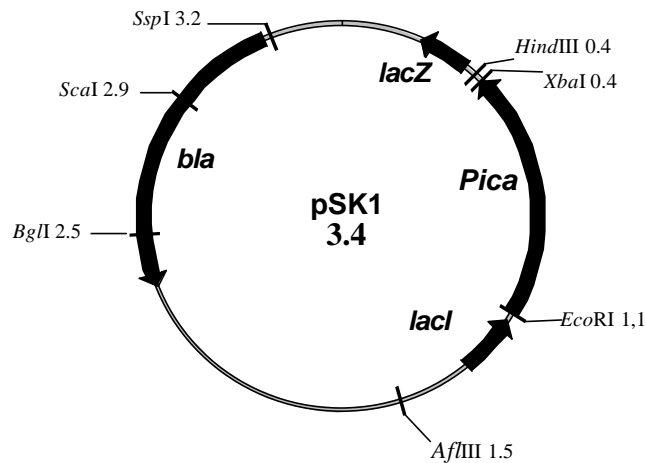
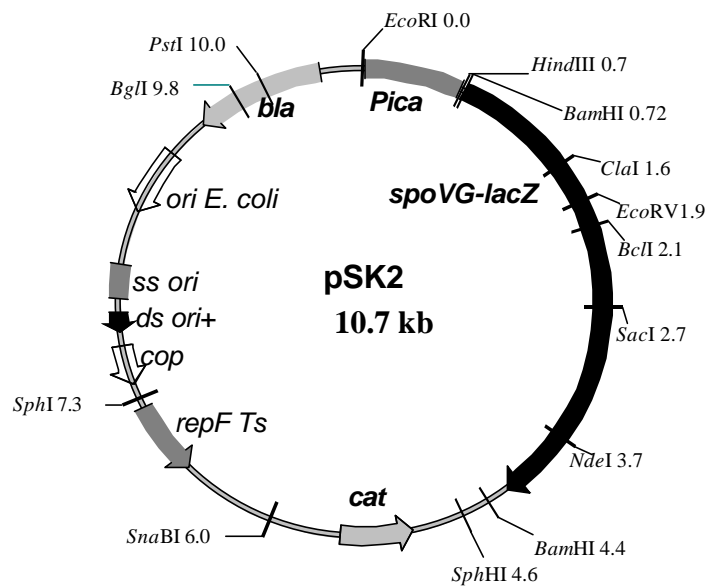


1. Restriction map of the plasmids used in this work

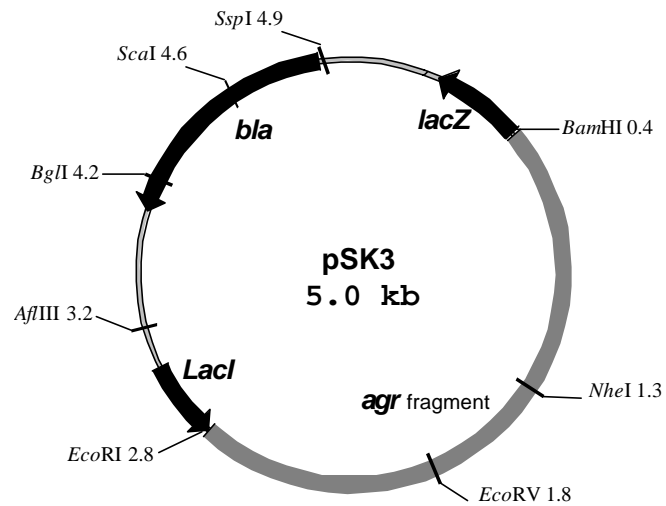
pSK1: an *EcoRI/XbaI* restricted, 0,7 kb PCR-product including the *ica* promoter cloned into an *EcoRI/XbaI* restricted vector pUC18.



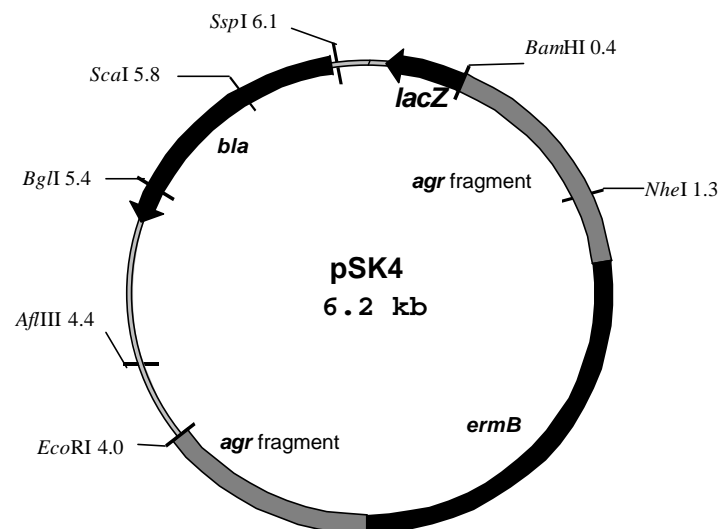
pSK2: The plasmid pSK1 is restricted with *EcoRI/HindIII*, and the 0,7 kb-PCR fragment including the *ica* promoter is cloned into the *EcoRI/HindIII* restricted pKO10 so as to obtain the $P_{ica}::lacZ$ fusion.



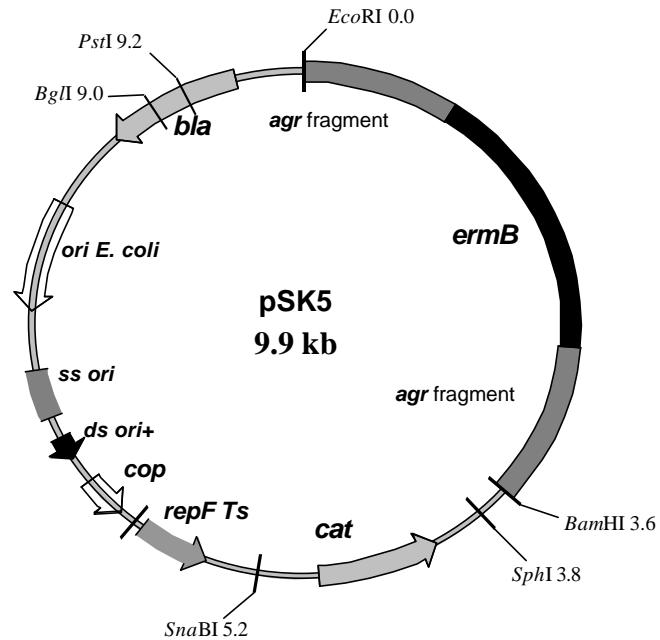
pSK3: an *EcoRI/BamHI* restricted 2395 bp PCR-fragment (including *AgrC*, D, B, P2, P3, and RNAIII of *S. epidermidis agr* system) cloned into an *EcoRI/BamHI* restricted pUC18.



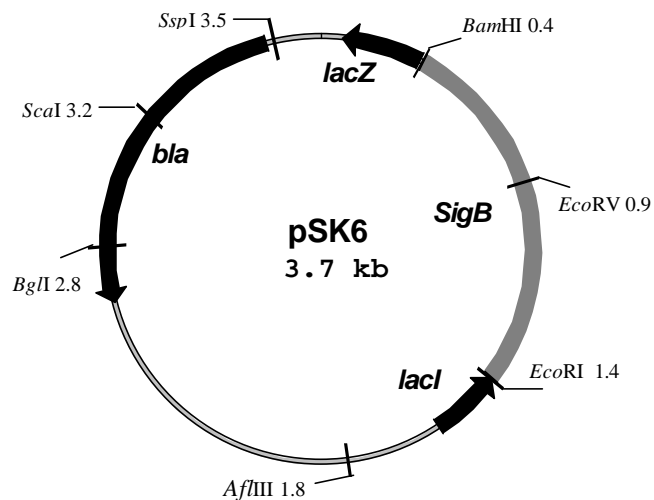
pSK4: a 1,7 kb blunt ended *ermB* gene cloned into a blunt ended, *NheI/EcoRV* restricted pSK3.



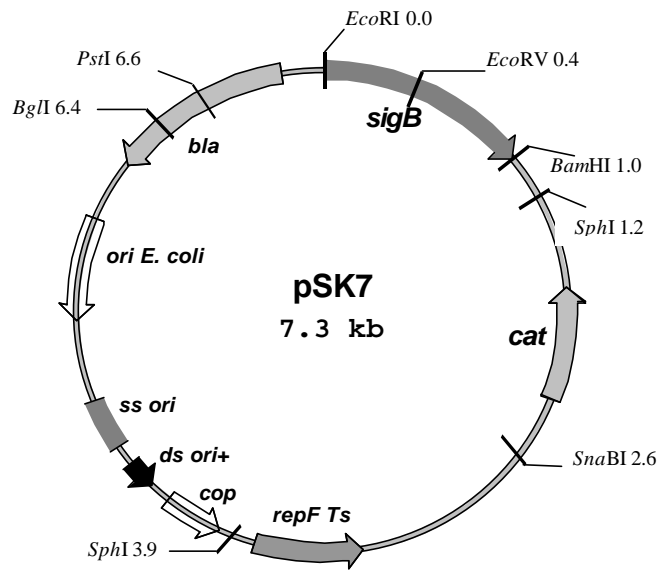
pSK5: A 3.6 kb *EcoRI/BamHI* restricted *agr::ermB* fragment from pSK4 cloned into an *EcoRI/BamHI* digested plasmid pKO10.



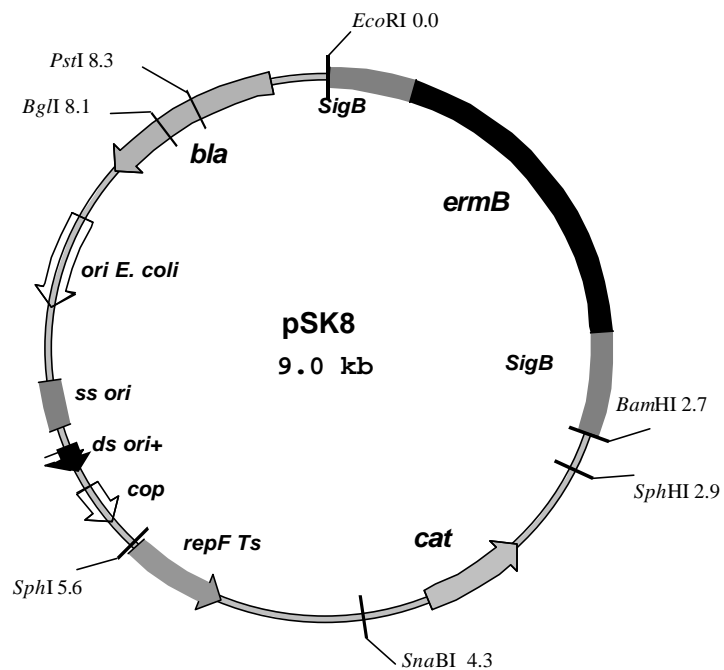
pSK6: A ~1,1 kb, *EcoRI/BamHI* digested *sigB* gene amplified by PCR cloned into an *EcoRI/BamHI* digested pUC18.



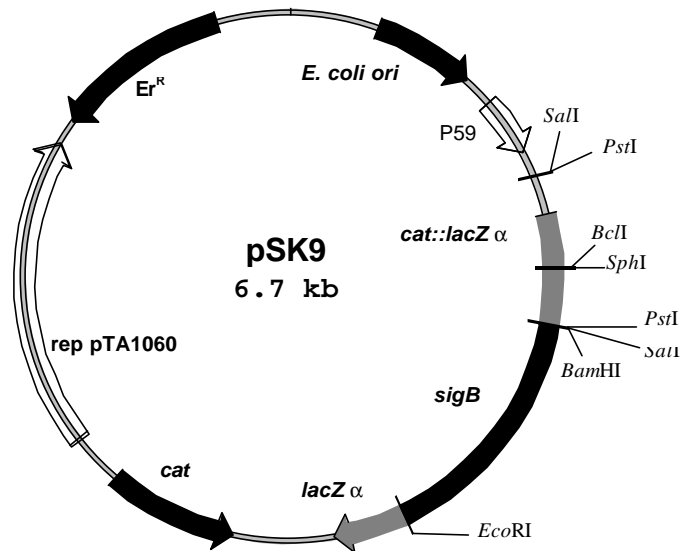
pSK7: A ~1,1 kb, *EcoRI/BamHI* digested *sigB* gene amplified by PCR cloned into an *EcoRI/BamHI* digested plasmid pKO10.



pSK8: Is constructed by cloning a blunt ended *ermB* cassette into the *sigB* gene in plasmid pSK7 after digestion with *EcoRV*.



PSK9: Is resulted from cloning of the *EcoRI/BamHI* digested *sigB* gene (PCR-fragment) into the multiple cloning site of the shuttle vector pHPS9, following digestion with *EcoRI* and *BamHI*.



2. Acronyms and abbreviations

<i>agr</i>	accessory gene regulator
Ap/Ap ^r	ampicillin/ampicillin resistant
BHI	brain heart infusionbroth
bp	base pairs
BSA	Bovine serum albomine
<i>cat</i>	chloramphenicol-acetyl-transferase
CIP	calf intestinal alkaline phosphatase
Cm/Cm ^r	chloramphenicol/ chloramphenicol resistant
dATP	desoxyadenosin-5´-triphosphate
dCTP	desoxycytosin- 5´-triphosphate
DEPC	diethyl pyrocarbonate
dGTP	desoxyguanosin- 5´-triphosphate
DNA	desoxyribonucleic acid
DNase	desoxyribonuclease
DTT	dithiothreitol
dNTP	desoxynucleotide
dTTP	desoxythymidin- 5´-triphosphate
EDTA	ethylene-diamin-tetraacetate
EtOH	ethanol
ELISA	enzyme-linked immunosorbent assay
g	gram
h	hour
HEPS	<i>N</i> -(2-hydroxyethyl)-piperazine- <i>N</i> '-(2-ethanesulfonic acid)
<i>hla</i>	α-toxin, gene cluster
<i>ica</i>	intercellular adhesin, gene cluster
IPTG	isopropyl-β-D-thiogalactoside
kb	kilobase (pairs)
kDa	kiloDalton
Km	kanamycin
l	liter
<i>lacZ</i>	β-galactosidase

LB	Luria bertani broth
M	molar
MCS	multiple cloning site
mg	milligram
MH	Müller-Hinton broth
MIC	minimal inhibitory concentration
min	minute
ml	milliliter
mM	millimeter
MOPS	3-(<i>N</i> -morpholino)propanesulfonic acid
MRSA	methicillin resistant <i>S. aureus</i>
MSSA	methicillin sensitive <i>S. aureus</i>
µg	microgram
µl	microliter
ng	nanogram
OD	optical density
ON	overnight
ORF	open reading frame
ori	origin of replication
PAE	post antibiotic effect
PBS	phosphate buffered saline
PCR	polymerase chain reaction
RBS	ribosome binding site
RLU	relative light unit
RNA	ribonucleic acid
RNase	ribonuclease
rpm	rotation per minute
RT	room temperature
<i>sar</i>	Staphylococcal accessory regulator
SD	Shine-Dalgarno
SDS	sodium-dodecyl-sulfate
SSC	standard saline citrate
t	time
TAE	Tris-acetate-EDTA

Tc	tetracycline
TE	Tris-EDTA
TPE	Tris-phosphate-EDTA
Tris	Tris-(hydroxymethyl)-aminomethan
TSB	tryptic soyabean broth
TSST-1	toxic shock syndrome toxin-1
UV	ultraviolet irradiation
V	volt
vol/vol	volume/volume
W	watt
WT	wild type
wt/vol	weight/volume
X-Gal	5-bromo-4-chloro-3-indolyl- β -glucoside

3. Publications and Presentations

- **Publications**

- 1.1. Rachid, S., S. Cho, K. Ohlsen, J. Hacker, and W. Ziebuhr. 2000. Induction of *Staphylococcus epidermidis* biofilm formation by environmental factors: The possible involvement of the alternative transcription factor sigB. (in press). In L. Emödy, G. Blum-Oehler, J. Hacker, and T. Pal (eds.), Genes and proteins underlying microbial urinary tract virulence: Basic aspects and applications. Advances in Experimental Medicine and Biology. Plenum Press, New York.
- 1.2. Rachid, S., K. Ohlsen, W. Witte, J. Hacker, and W. Ziebuhr. 2000. Effect of subinhibitory antibiotic concentrations on the polysaccharide intercellular adhesin (PIA) expression in biofilm forming *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. (submitted)
- 1.3. Rachid, S., K. Ohlsen, M. Hecker, J. Hacker, and W. Ziebuhr. 2000. The role of the alternative sigma factor *sigB* in biofilm regulation in *Staphylococcus aureus*. J. Bacteriol. (submitted)
- 1.4. Ziebuhr, W., V. Krimmer, S. Rachid, I. Löbner, F. Götz, and J. Hacker. 1999. A novel mechanism of phase variation of virulence in *Staphylococcus epidermidis*: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. Mol. Microbiol. 32 (2): 345-356.

- **Meetings and presentations**

- 2.1. Rachid, S., W. Ziebuhr, K. Ohlsen, and J. Hacker. Use of *lacZ* as a reporter gene to investigate the *ica* gene expression in biofilm positive *Staphylococcus epidermidis*. VAAM / DGHM-Spätsommerschule (Usedom) “ Pathogenitätsmechanismen” Zinnowitz, 1 - 3 October, 1998.
- 2.2. Rachid, S., W. Ziebuhr, K. Ohlsen, and J. Hacker. Use of *lacZ* as a reporter gene to investigate the *ica* gene expression in biofilm positive *Staphylococcus epidermidis*. DGHM-Kongress Berlin „ Mikrobielle Evolution & Infektion“ Berlin, 4 - 9 October, 1998.
- 2.3. Rachid, S., W. Ziebuhr, K. Ohlsen, and J. Hacker. Effect of environmental factors on *ica* expression in biofilm forming *Staphylococcus epidermidis*. Statusseminar Graduiertenkollegs “Infektiologie” Universität Würzburg und “Infektion und Immunität der Ludwig-Maximilians-Universität München” Herrsching, 04 - 06 Dezember 1998.
- 2.4. Rachid, S., K., Ohlsen, Seong-Hag-Cho, J., Hacker, and W., Ziebuhr. Influence of antibiotics, environmental factors and internal regulatory signals on the polysaccharide intercellular adhesin (PIA) expression in biofilm forming *Staphylococcus epidermidis*. FEMS symposium “Genes and proteins underlying microbial urinary tract virulence” - Basic aspects and applications. Pécs, Hungary, 16 - 19 September, 1999.
- 2.5. Rachid, S., W. Ziebuhr, K. Ohlsen, and J. Hacker. Influence of antibiotics, environmental factors and internal regulatory signals on the polysaccharide intercellular adhesin (PIA) expression in biofilm forming *Staphylococcus epidermidis*. Treffen der Graduiertenkollegs München und Würzburg. Kloster Banz, 27 - 29 October, 1999.
- 2.6. Rachid, S., W. Ziebuhr, K. Ohlsen, and J. Hacker. Effect of environmental stresses and the role of *sigB* in regulation of biofilm in *Staphylococcus epidermidis*. Workshop “Signalketten und Mikrobielle Pathogenität: Von der Biochemie bis zur Medizin”, Lutherstadt-Wittenberg, 04 - 06 November 1999.

4a. Curriculum vitae

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1989 – 1992 M.Sc. in biology - College of science, University of Salahaddin-Irak. The title of the thesis: Study on the antibiotics production by some Streptomyces isolates with a partial identification of these antibiotics

1992 – 1996 Assistant teacher - college of Medicine, University of Sulaimaniah - Irak

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1989 – 1992 „M.Sc.“ in der Biologiefakultät an der Universität Salahaddin-Irak

1992 – 1996 Assistentslehrer an der Medizinischen Fakultät - Universität Sulaimaniah - Irak

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