molecular cloning and DNA sequencing. J. Bacteriol. **178**:6036-6042.

37. **Zukowski, M. M., D. F. Gaffney, D. Speck, M. Kauffmann, A. Findeli, A. Wisecup, and J. P. Lecocq.** 1983. Chromogenic identification of genetic regulatory signals in *Bacillus subtilis* based on expression of a cloned *Pseudomonas* gene. Proc. Natl. Acad. Sci. USA. **80**:1101-1105.

FIGURE LEGENDS

Fig.1 Mapping of *fmtA* region of *S. aureus* KSA8. The arrows represent the ORFs and the direction of transcription. The box represents the FmtA protein, indicating the four motifs, which are typically found in PBPs and ß-lactamases.

Fig.2 Site directed mutagenesis of FmtA protein. Site directed mutagenesis and complementation test (MIC measurement) were performed as described in Materials and Methods. White letters represent the mutagenesis site of amino acid residues.

Fig.3 Muropeptide pattern of strain KSA8, its mutants KSA8-TS339 and KSA8-TS339C (*cat::fmtA*). Cell walls were isolated and digested with muramidase and subjected to reversed-phase HPLC. For each strain an overview of the whole elution profile (10-220 minutes, diagrams on the right side of the Fig.) and a more detailed view of the region between 10 and 90 minutes retention time containing mostly the monomeric region (left hand side of the Fig.) are given. The corresponding strains are indicated in each separate graph. For the first complete chromatogram in the upper right corner the areas corresponding to muropeptide monomers (no peptide crosslinking), dimers (one crosslink), trimers (two crosslinks) etc. are indicated. The numbered peaks in the left hand side of the Fig. correspond to the following muropeptides (for nomenclature see text and ref.

20, 25): 1=M1, 2=M2*, 3=M6*, 4=M2, 5=M3, 6=M4 (main monomer), 7=M9 (non-amidated M4), 8=M10, 9=D2*, 10=D3 (dimer of M4).

Fig.4 Western blot of fractions from *S. aureus* COL. Fractionation method is described in Materials and Methods. Samples were prepared from COL (lane A, D-G), COL-TS339C (lane B), and COL-TS339 integrated pHK4156 (lane C), and resolved by SDS-PAGE in 10% polyacrylamide gel, then subjected to Western blotting. Anti-FmtA serum was used for primary serum. FmtA band was marked with *. Lanes: A-C, whole cell lysate; D, culture supernatant; E, membrane extract; F, cell wall extract; G, cytoplasmic extract; H, recombinant FmtA.

Fig.5 XylE activity of *fmtA* promoter of COL during growth. Preparation of samples and XylE assay were described in Material and Methods. Solid squares and circles represent OD_{660} of cultures and XylE activity, respectively.

Fig.6 XylE activity of *fmtA* promoter in the presence of antibiotics. Preparation of samples for XylE assay and XylE assay were described in Materials and Methods. Bars represent 1 SD. *, $0.01<P<0.05$; **, $P<0.01$.

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Origin or reference
Strains		
S. aureus		
KSA8	homogeneous Mc^r	(13)
COL	homogeneous Mc^r	(13)
RN4220	8325-4r-	R. Novick
RN4220 TS339C	Mutagenized *fmtA*::*cat*	This study
COL TS339	Mutagenized *fmtA*::Tn*551*	(13)
COL TS339C	*fmtA*::*cat* gene (Transduction from RN4220 TS339C)	This study
KSA8 TS339	*fmtA*::Tn*551* (Transduction from COL TS339)	(13)
KSA8 TS339C	*fmtA*::*cat* gene (Transduction from RN4220 TS339C)	This study
BB270	heterogeneous Mc^r	(13)
BB270 TS339	*fmtA*::Tn*551* (Transduction from COL TS339)	(13)
HK9605	pHK4147/COL	This study
HK9610	pHK4125/COL	This study
HK9710	pHK4080/RN4220	This study
E. coli		
XL-1 Blue	*rec*A1 *end*A1 *gyr*A96 *thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI*^{q}*ZM15* Tn*10* (Tet^r)]	(2)
HMS174	*rec*A1 *hsdR* rif^r	(23)
HK4172	HMS174/pHK4171	This study
Plasmids		
pCL52.1	shuttle vector (thermosensitive), SP^r in *E. coli*, Tc^r in *S. aureus*	(28)
pCL83	shuttle vector, Amp^r in *E. coli*, Tc^r in *S. aureus*	(13)
pLI50	shuttle vector, Amp^r in *E. coli*, Cm^r in *S. aureus*	(3)
pGEM T-easy	PCR cloning vector, Amp^r in *E. coli* (Promega)	
pET28C	expression vector (Novagen)	
pSL24	promoter less *xylE* gene for reporter system	(26)
pHK4033	pUC19/3.6 kb *Hin*dIII fragment of KSA8	This study
pHK4079	pCL52.1/3.5 kb *Eco*RI-*Sal*I fragment from pHK4033	This study
pHK4080	pCL52.1/3.5 kb *Eco*RI-*Sal*I fragment + *cat* gene	This study
pHK4130	pGEM-T easy/1.2 kb PCR fragment (*fmt*A) of KSA8	This study
pHK4156	pCL83/1.4 kb *fmtA* of KSA8	This study
pHK4188	pCL83/mutagenized *fmtA* (46S→46C)	This study
pHK4157	pCL83/mutagenized *fmtA* (63S→63C)	This study
pHK4159	pCL83/mutagenized *fmtA* (127S→127C)	This study
pHK4186	pCL83/mutagenized *fmtA* (186Y→186C)	This study
pHK4171	pET28c/*fmtA* of KSA8	This study
pHK4125	pSL24/*fmtA* promoter region (*BamH*I-*Hin*dIII fragment)	This study
pHK4147	pSL24/*orf1* promoter region (*BamH*I-*Eco*RI fragment)	This study

Table 2. MIC of oxacillin of *fmtA*-inactivated mutants and their parents

strain	MIC (μg/ml)	strain	MIC (μg/ml)
KSA8	512	COL	512
KSA8-TS339	16	COL-TS339	64
KSA8-TS339C	16	COL-TS339C	64

Table. 3 Synthetic primers used for site-directed mutagenesis

	46 C D N 48
primer 1	5'-CAAAATTATGTGACAATGAAAAATATTTAG-3'
primer 2	3'-GTATTCCTTTGTTTTAATACACTGTTACTT-5'
	63 C K L K 66
primer 3	5'-GTTGCGCCGTGTAAACTAAAAGAGGTATAT-3'
primer 4	3'-ATTCCTTTTCCAACGCGGCACATTTGATTT-5'
	127 C A Q K 130
primer 5	5'-TTTTAATAGGTTGTGCTCAAAAATTTTCAA-3'
primer 6	3'-TTATGCTACAAAAATTATCCAACACGAGTT-5'
	186 C K N 188
primer 7	5'-CAAAAGATTGTAAAAATTTAGATCAAGCAG-3'
primer 8	3'-ATATTTAGGAGTTTTCTAACATTTTTAAAT-5'

Table 4. Cell wall composition from HPLC data

strain	% Monomers	% Dimers	% Trimers	% Oligomers	% Crosslinking	Ratio M4/M9
KSA8	5.4	8.5	7.7	78.4	80	3.5
KSA8-TS339	7.9	11.7	11.0	69.4	75.5	3.1
KSA8-TS339C	8.2	11.7	10.4	69.7	74.9	1.7

Table 5. XylE activity in the presence of ß-lactam antibiotics

oxacillin concentration (μg/ml)	XylE activity (mU / mg)	
	fmtA	*orf1*
none	14.8 ± 1.4[a]	20.9 ± 0.9
1	17.0 ± 1.4	21.8 ± 1.3
10	22.6 ± 2.3	21.1 ± 1.6
100	42.8 ± 3.5	18.2 ± 0.6

a: mean + S.D.

Table 6. Effect of oxacillin on the amount of FmtA

oxacillin concentration (μg/ml)	relative density [a]	
	FmtA	62kDa-AM
none	1.0	1.0
1	1.42 ± 0.19 [b]	1.09 ± 0.08
10	1.68 ± 0.07	1.05 ± 0.04
100	2.03 ± 0.23	0.99 ± 0.03

a: The amount of FmtA or 62 kDa-AM was measured by scanning densitometry of the blot and the relative density was calculated as a ratio of density of protein in the experimental sample to that in a sample prepared from the strain grown in the absence of oxacillin.
b: mean ± S.D.

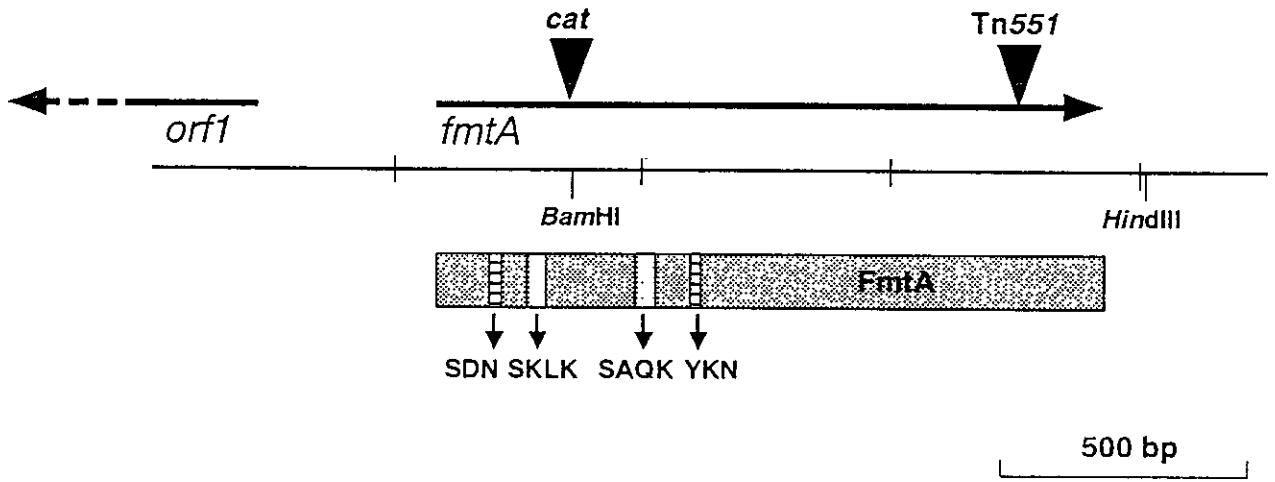
cat
Tn551
orf1
fmtA
BamHI
HindIII
FmtA
SDN SKLK SAQK YKN
500 bp

Fig. 1

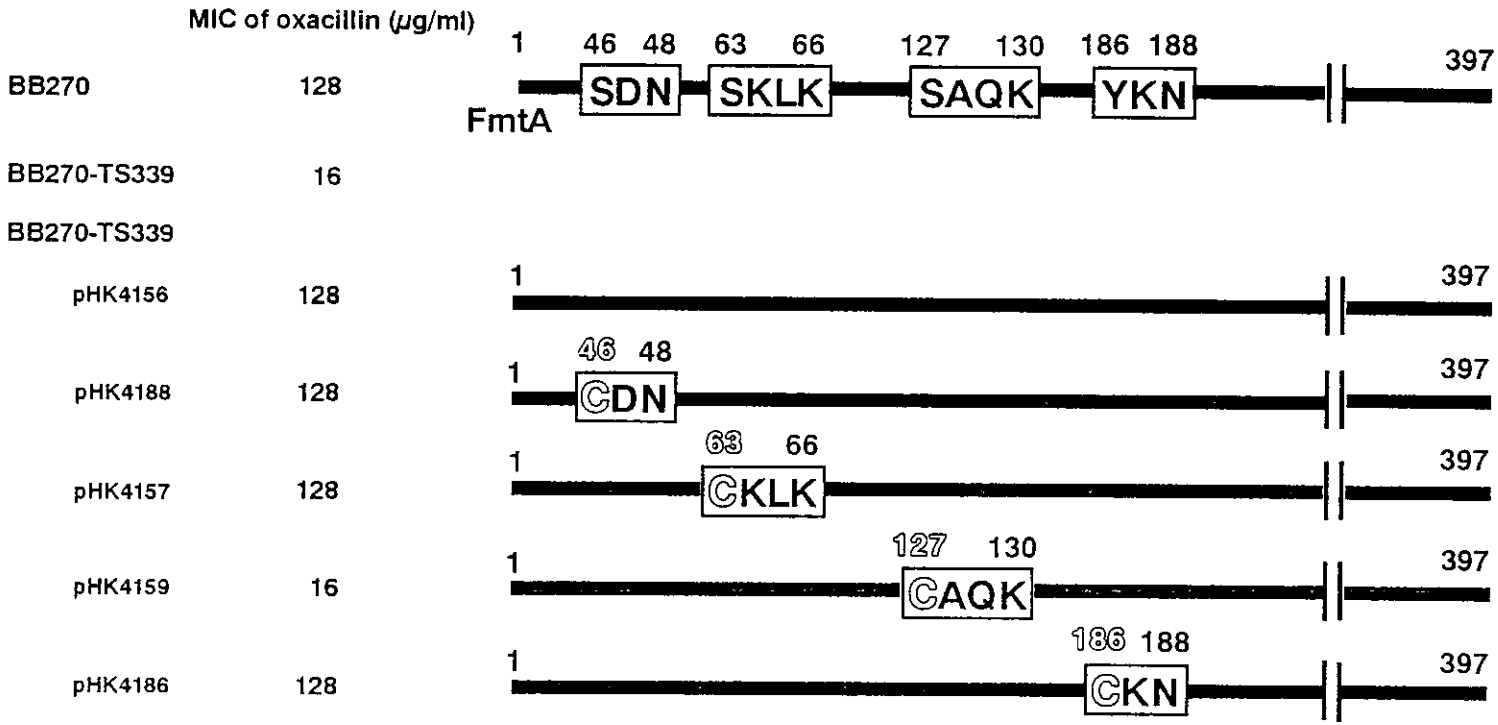
MIC of oxacillin (μg/ml)
BB270
128
BB270-TS339
16
BB270-TS339
pHK4156
128
pHK4188
128
pHK4157
128
pHK4159
16
pHK4186
128
FmtA
1
46 48
63 66
127 130
186 188
397
SDN
SKLK
SAQK
YKN
CDN
CKLK
CAQK
CKN

Fig. 2

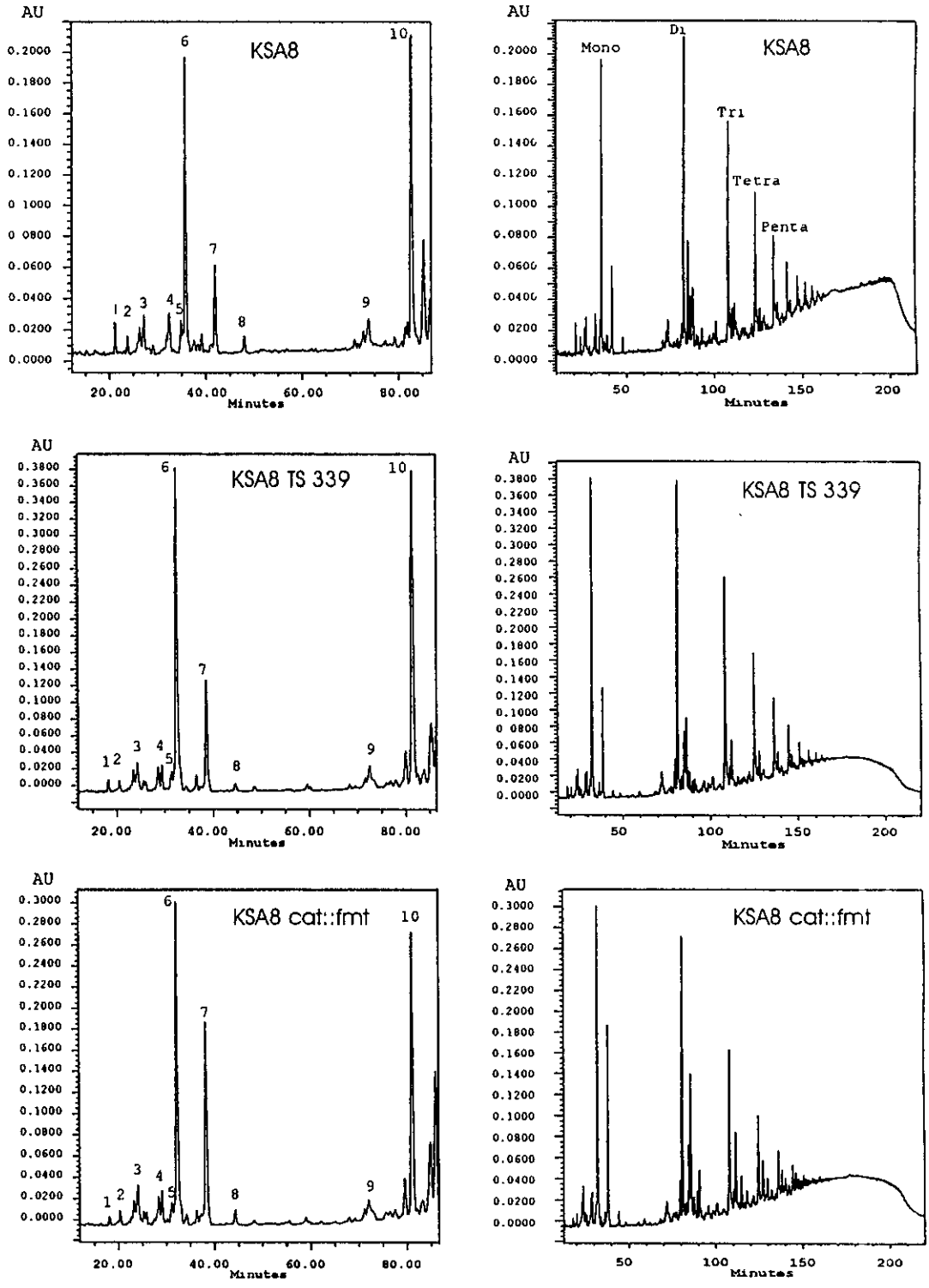
AU
KSA8
Minutes
AU
Mono
Di
Tri
Tetra
Penta
KSA8
Minutes
AU
KSA8 TS 339
Minutes
AU
KSA8 TS 339
Minutes
AU
KSA8 cat::fmt
Minutes
AU
KSA8 cat::fmt
Minutes

Fig. 3.

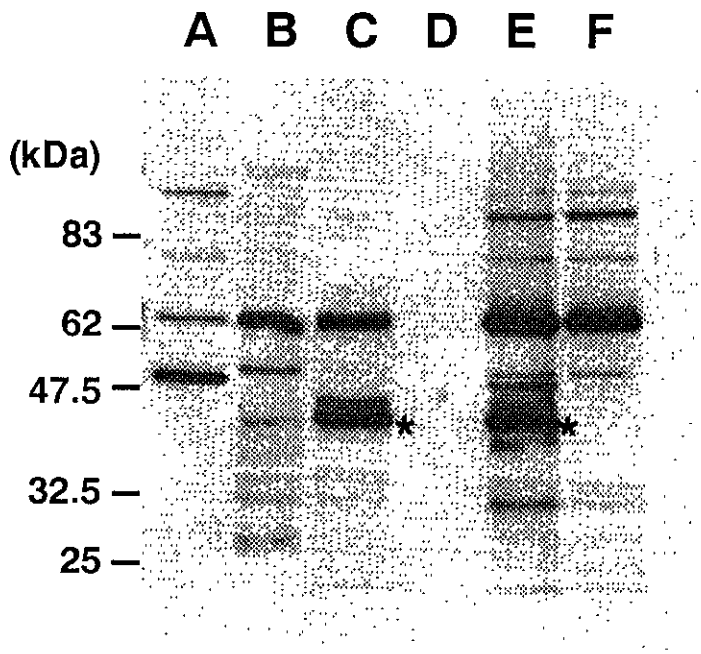

Fig.4

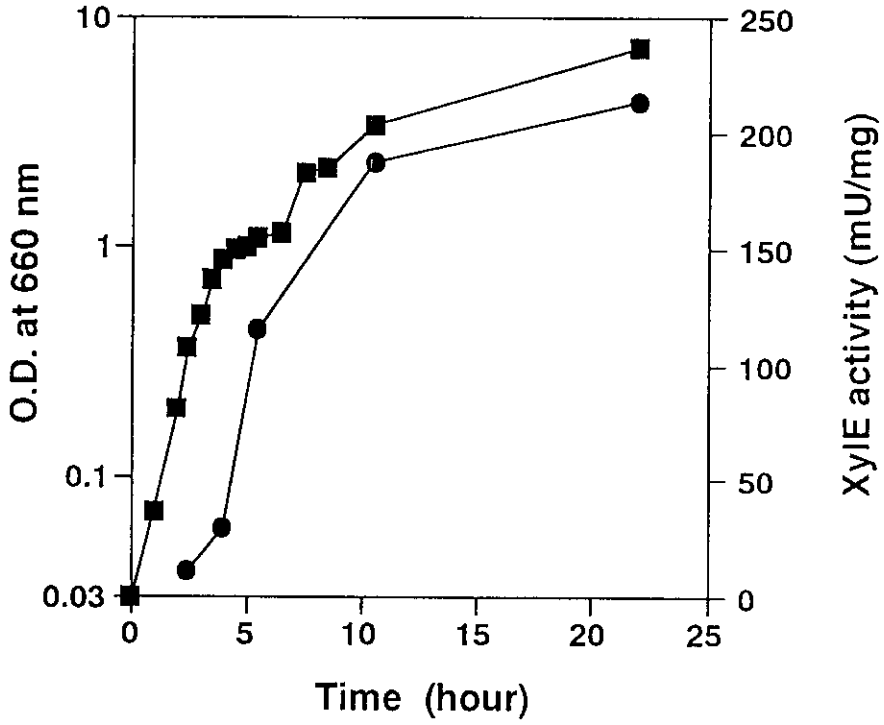
10
1
0.1
0.03
O.D. at 660 nm
250
200
150
100
50
0
XylE activity (mU/mg)
0
5
10
15
20
25
Time (hour)

Fig. 5

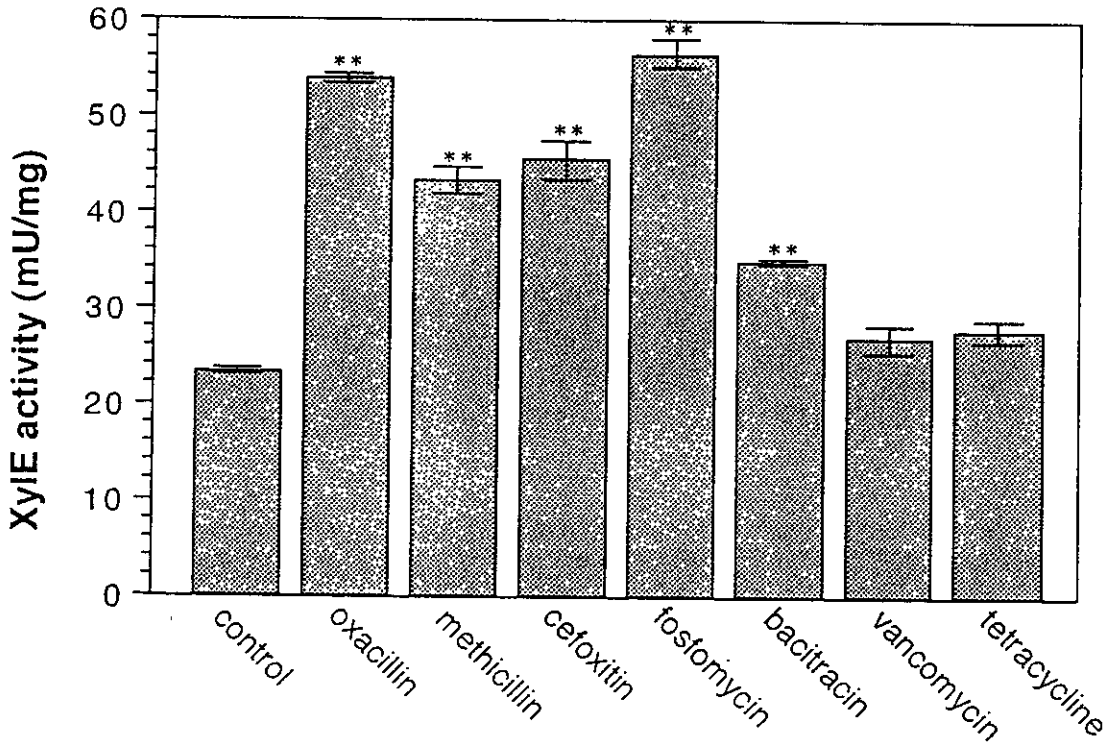
XylE activity (mU/mg)
60
50
40
30
20
10
0
**
**
**
**
**
control
oxacillin
methicillin
cefoxitin
fosfomycin
bacitracin
vancomycin
tetracycline

Fig. 6