Supporting information data legend

Fig. S1

Bio-informatic analysis of PBPs

A - Alignment of the proposed active sites of HMT Class 1 PBPs that have defined active sites compared to PBP 2B, SpoVD and PBP 3 of *Bacillus subtilis*. The position of the active site serine residue for PBP 2B and PBP 3 is indicated at the top and bottom of the alignment.

B – Pairwise comparison of PBPs encoded in the *Bacillus* genome. Each box shows the percentage identity and similarity (in parenthesis) for that pair of PBPs along with the region of overlap (including gaps) analysed. The values are shown in red represent PBPs that show significant similarity(> 27.5 % identity) which in most cases, corresponds with PBPs known to have similar functions, if not functional redundancy e.g. PBP 2A and PBP H (Wei *et al*., 2003) and PBP1, PBP 2C, PBP 4 and PBP G (Popham and Setlow, 1996).

Fig S2

Immunofluorescent detection of PBP 3 and PBP 2B

Panel A: A Western blot image of total protein samples of vegetative 168 and 3105 cultures separated by SDS-PAGE using a polyclonal antiserum raised against full length PBP 3. The lanes from left to right correspond to samples of strain 168, and 3105 grown in PAB supplemented with various concentrations of xylose (0 - 0.5 %). The lower arrows corresponds to the position where wild-type PBP 3 would be expected to migrate and the upper arrow for a GFP-PBP 3 fusion.

Panel B: Characteristic images of cells stained for either PBP 3 or PBP 2B using polyclonal antisera that were used to derive "heat map" representations for the subcellular distribution of the PBPs in a cell population relative to cell length. Panels A and B show a typical cell staining patterns obtained for PBP 3 and PBP 2B respectively for the strains 168 and 4001 relative to cell morphology and DNA distribution.

Fig S3.

PBP profiles of strains with conditional expression of *pbpC*.

A - Analysis of oxacillin sensitivity for strains with conditional expression of *pbpC.*

Strains WT (168), ΔpbpC (4015), KS51, KS53, KS52, KS55, 4001, KS51 and KS54 were spotted out as horizontal sequence corresponding to a 1:4 dilution series of the cultures on plates that were incubated for 16 h before being photographed. (A) Nutrient agar alone, (B) Nutrient agar with Oxacillin (0.04μg/ml) and (C) Nutrient agar with oxacillin (0.04 μg/ml) and IPTG (1mM).

B - A fluorogram of an SDS-page gel showing the PBP profile of strains (168, 4001, 4015, KS50, KS51, KS53, KS54, KS52 and KS55 form left to right) sampled in mid-exponential growth in the presence or absence of IPTG (1 mM) and labelled with Bocillin-FL as described in the methods. The relevant genotype of each strain is shown above each lane of the gel. The arrows mark the location of bands corresponding to PBP 2B (1) and PBP 3 (2) that either change in intensity or are absent as expected for the genotype or growth conditions.

Fig. S4.

Aligment of PBP 3, PBP 2B, PBP 2x, PBP 2A

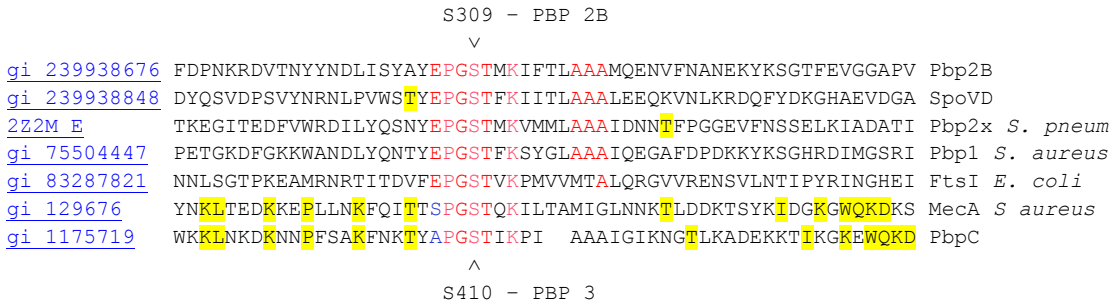
Alignment of PBP 3 to class B PBPs from *S. aureus* (PBP 2A), *B. subtilis* (PBP 2B), *S. pneumonia* (PBP 2X) and *E. coli* (PBP 3) using Uniport database. Sequence similarity was highlighted in three colours blue, red and green ranging from low to high sequence similarity. The N-terminus of PBP 3, which consist of the MecA domain (residues 26-147), showed high similarity only to *Sa*PBP 2A, whereas the dimerization domain (residues 155-322) and the TPase domain (residues 352-666) of PBP 3 showed comparable similarity to all class B PBPs.

Fig. S5

Both PBP 3 and SigM regulated genes act to confer β–lactam resistance. To determine the contributions of *sigM* and *pbpC* to resistance to cell wall synthesis inhibitors exponentially growing cultures of strains 168 (wild type), RD300 (*sigM*), 4015 (*pbpC)*, RD301(*pbpC sigM*), and BSU2007 (Δ7 ECF), were serially diluted and spotted onto plates containing moenomycin (A; 8 μg/ml), Penicillin G (B; 0.005 μg/ml), Cephalexin (C; 0.08 μg/ml), Oxacillin (D; 0.04 μg/ml), and no antibiotic (E). Plates were then incubated at 37 °C for 18 h prior to being photographed

Fig. S1

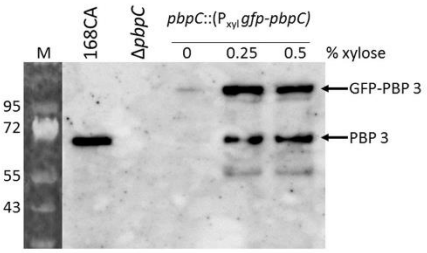
A



B

PBP protein	PBP 1A	PBP 2A	PBP 2B	PBP H	PBP 2C	PBP 3	PBP 4	PBP 4*	PBP G	SpoVD	PBP I	PBP X
PBP 1A (914aa)												
PBP 2A (713aa)	25.1% (53.3%) 375											
PBP 2B (716aa)	24.1% (54.8%) 294	24.3% (54.0%) 643										
PBP H (685aa)	-	42.8% (74.7%) 703	23.8% (53.4) 562									
PBP 2C (714aa)	33.2% (65.2%) 742	-	-	-								
PBP 3 (668aa)	-	24.4% (54.8%) 610	27.2% (54.9%) 541	-	26.9% (51.6%) 335							
PBP 4 (624aa)	31.8% (64.0%) 606	-	-	-	33.5% (64.4%) 576	24.6% (50.8%) 382						
PBP 4* (451aa)	-	-	-	-	-	27.0% (51.3%) 337	-					
PBP G (647aa)	31.7% (63.3%) 665	-	24.3% (54.2%) 354	22.8% (49.7%) 382	33.8% (66.2%) 625	23.0% (51.8%) 330	30.0% (62.5%) 594	-				
SpoVD (645aa)	-	27.2% (54.2%) 570	33.9% (63.7%) 641	26.5% (56.6%) 449		27.7% (55.9%) 538	23.4% (50.6%) 427	-	22.5% (51.2%) 365			
PBP I (584aa)	25.4% (53.2%) 327	22.1% (56.6%) 516	24.2% (55.5%) 591	25.0% (55.1%) 508	22.8% (51.8%) 556	23.2 (53.5%) 565	-	-	-	27.9% (55.3%) 591		
PBP X (391aa)	-	-	-	-	-	-	-	31.3% (59.9% 319	-	-	-	

A



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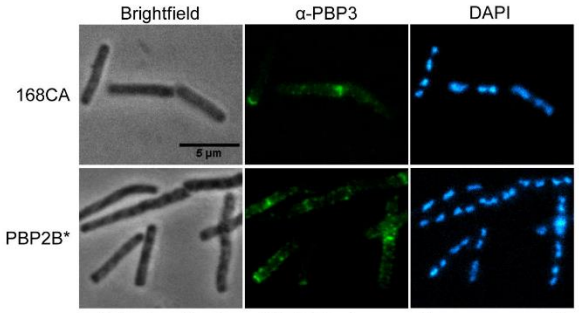
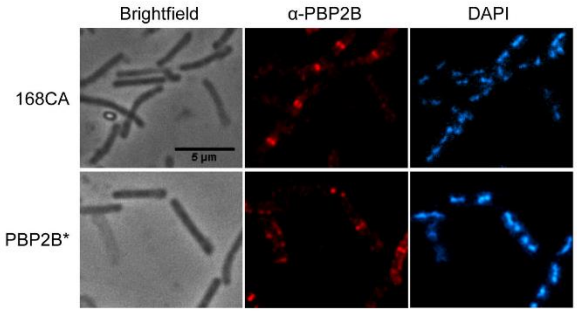


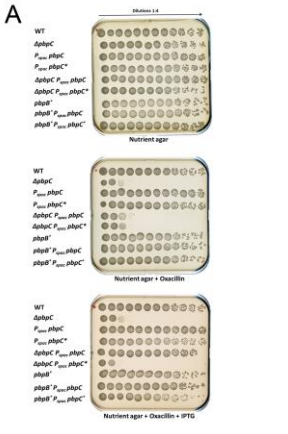
Cellular localization of PBP3 by immunofluorescence using α-PBP3 antibody

C



Cellular localization of PBP2B by immunofluorescence using α-PBP2B antibody

Fig S2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pbpB'
pbpB' P_spac pbpC
pbpB' P_spac pbpC*
Nutrient agar
Nutrient agar + Oxacillin
Nutrient agar + Oxacillin + IPTG
B
WT
pbpB'
ΔpbpC
P_spac pbpC
pbpB' P_spac pbpC
P_spac pbpC*
pbpB' P_spac pbpC*
ΔpbpC P_spac pbpC
ΔpbpC P_spac pbpC*
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PBP 2B
PBP 2X
PBP 3
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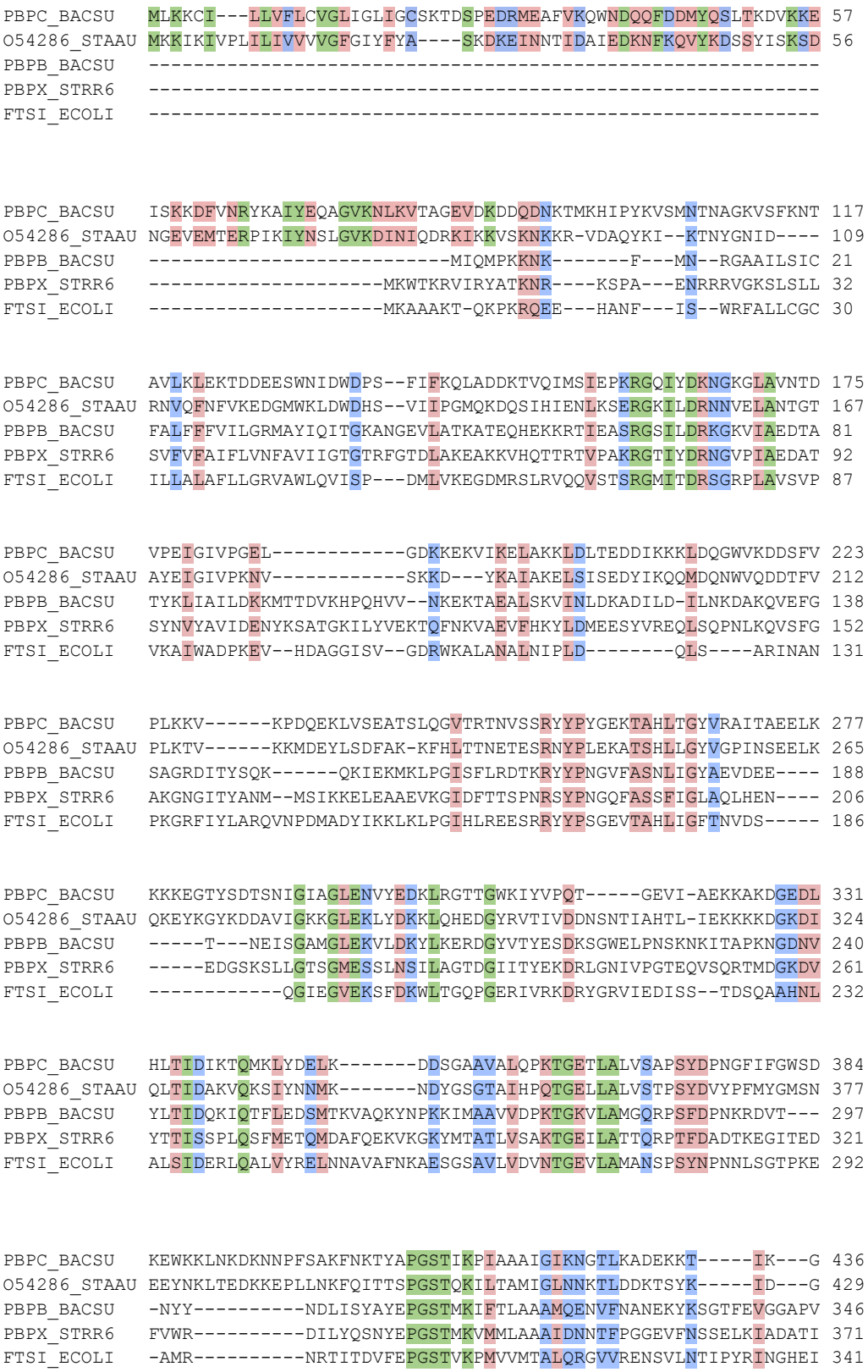
PBP 5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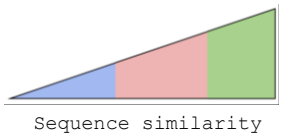
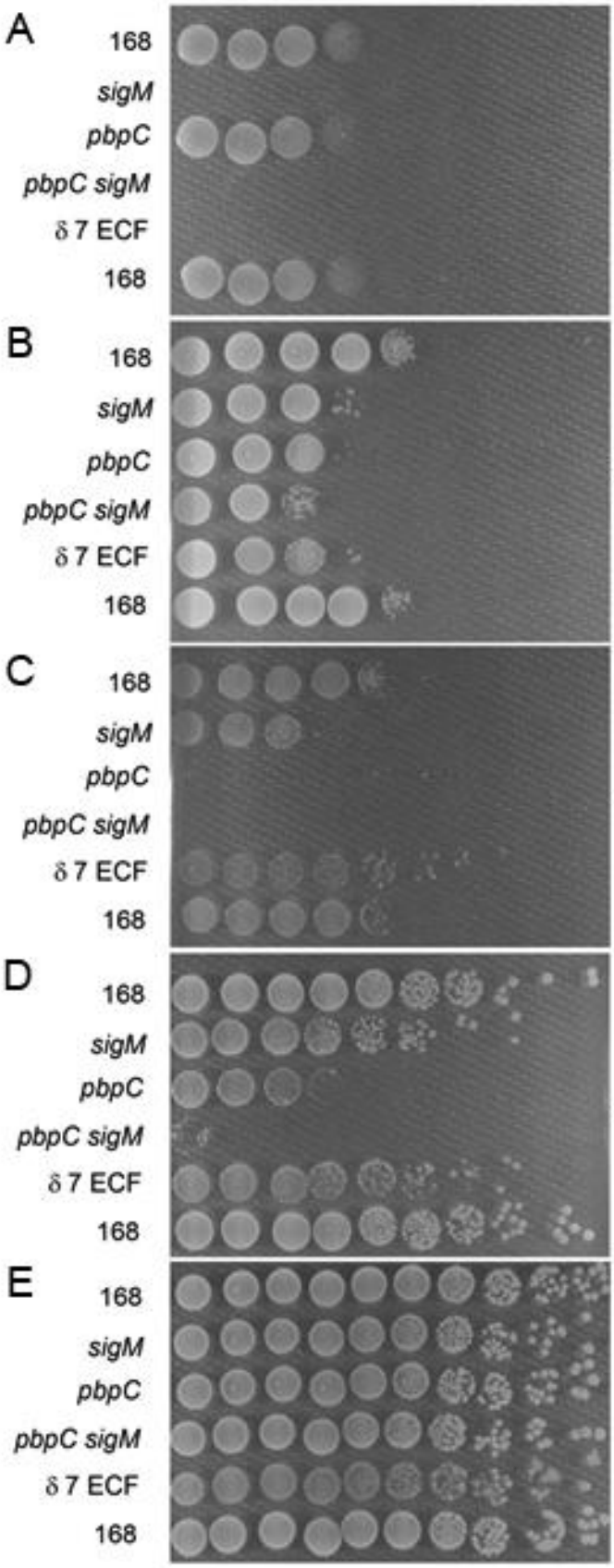


Fig S4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Control

Fig S5