Table S1. Strains and plasmids used or constructed in this work

Strain or Plasmid	Genotype/relevant characteristics	Reference or source
P. aeruginosa		
PAO1	Reference strain fully sequenced	Laboratory collection
PAOΔ*ampC*	PAO1 Δ*ampC*::*lox*	(1)
PAOΔ*dacB*	PAO1 Δ*dacB*::*lox*	(2)
PAOΔ*dacC*	PAO1 Δ*dacC*::*lox*	This study
PAOΔ*pbpG*	PAO1 Δ*pbpG*::*lox*	This study
PAOΔ*dacB*Δ*dacC*	PAO1 Δ*dacB*::*lox* Δ*dacC*::*lox*	This study
PAOΔ*dacB*Δ*pbpG*	PAO1 Δ*dacB*::*lox* Δ*pbpG*::*lox*	This study
PAOΔ*dacC*Δ*pbpG*	PAO1 Δ*dacC*::*lox* Δ*pbpG*::*lox*	This study
PAOΔ*dacB*Δ*dacC*Δ*pbpG*	PAO1 Δ*dacB*::*lox* Δ*dacC*::*lox* Δ*pbpG*::*lox*	This study
PAOΔ*dacB*Δ*ampC*	PAO1 Δ*dacB*::*lox* Δ*ampC*::*lox*	This study
PAOΔ*dacC*Δ*ampC*	PAO1 Δ*dacC*::*lox* Δ*ampC*::*lox*	This study
PAOΔ*pbpG*Δ*ampC*	PAO1 Δ*pbpG*::*lox* Δ*ampC*::*lox*	This study
PAOΔ*dacB*Δ*dacC*Δ*ampC*	PAO1 Δ*dacB*::*lox* Δ*dacC*::*lox* Δ*ampC*::*lox*	This study
PAOΔ*dacB*Δ*pbpG*Δ*ampC*	PAO1 Δ*dacB*::*lox* Δ*pbpG*::*lox* Δ*ampC*::*lox*	This study
PAOΔ*dacC*Δ*pbpG*Δ*ampC*	PAO1 Δ*dacC*::*lox* Δ*pbpG*::*lox* Δ*ampC*::*lox*	This study

PAOΔ*dacB*Δ*pbpG*Δ*ampC*Δ*dacC*	PAO1 Δ*dacB*::*lox* Δ*pbpG*::*lox* Δ*ampC*::*lox* Δ*dacC*::*lox*	This study
PAOΔ*dacB*Δ*dacC*Δ*pbpG*Δ*ampC*	PAO1 Δ*dacB*::*lox* Δ*dacC*::*lox* Δ*pbpG*::*lox* Δ*ampC*::*lox*	This study
E. coli		
XL1-Blue	F´::Tn*10 proA+proB_+lacI*^{q} Δ (*lacZ*)M15/*recA1 endA1 gyrA96* (Nal^{r}) *thi hsdR17* (r_k + m_k +) *mcrB1*	Laboratory collection[b]
S17.1λpyr	recA pro (RP4-2Tet::Mu Kan::Tn7)	Laboratory collection[b]
Plasmids		
pEX100Tlink	Ap^{r} *sacB*, pUC19-based gene replacement vector with an MCS	(3)
pUCGmlox	Apr Gm^{r}, pUC18-based vector containing the lox-flanked aacC1 gene	(3)
pCM157	Tc^{r}, *cre* expression vector	(3)
pEXTΔ*ampC*::Gm	pEX100Tlink containing 5´and 3´ flanking sequence of *ampC*::Gm *lox*	(1)
pEXTΔ*dacB*::Gm	pEX100Tlink containing 5´and 3´ flanking sequence of *dacB*::Gm *lox*	(2)
pEXTΔ*dacC*::Gm	pEX100Tlink containing 5´and 3´ flanking sequence of *dacC*::Gm *lox*	This study
pEXTΔ*pbpG*::Gm	pEX100Tlink containing 5´and 3´ flanking sequence of *pbpG*::Gm *lox*	This study

Table S2: Primers used for PCR amplification of both upstream (PCR1) and downstream (PCR2) regions to PAO1 genes: *pbpG, dacC, dacB* and *ampC*.

Target gene	Primer´s Name	Primer´s Sequence, 5´…3´[a]	PCR Product - bp	Reference
pbpG	pbpG-F1-ERI	-TCGAATTCCACTTTCAAAGCCCTACGTGC-		This study
	pbpG-RI- HDIII	-TCAAGCTTGCAGTTTCGAGTCGAGCACG-	PCR1= 375	
	pbpG-F2-HDIII	-TCAAGCTTCACTGCGATCCCGGCCGC-		
	pbpG-R2-BHI	-TCGGATCCCAGTACCGGACCCAGGAGC-	PCR2= 318	
dacC	dacCF-ERI	-TCGAATTCACCTTGGCCAGCCGACGC-		This study
	dacCIR-HDIII	-TCAAGCTTCTCGGCCAGGGCGACGC-	PCR1= 500	
	dacCIF-HDIII	-TCAAGCTTGAAGTGAAAGCCGGCCTCG-		
	dacCR-BHI	-TCGGATCCACGCTCGCAGGGGAATTCG-	PCR2= 400	
dacB	dacB-F1-ERI	-TCGAATTCCGACCATTCGGCGATATGAC-		(2)
	dacB-R1-HDIII	-TCAAGCTTGTCGCGCATCAGCAGCCAG-	PCR1= 571	
	dacB-F2-HDIII	-TCAAGCTTGCCAGGGCAGCGTACCGC-		
	dacB-R2-BHI	-TCGGATCCCGCGTAATCCGAAGATCCATC-	PCR2= 693	
ampC	AmpC-F-ERI	-TCGAATTCGCGCGCAGGGCGTTCAG-		(1)
	AmpC-I-R-HDIII	-TCAAGCTTCGTCCTCTTACGAGGCCAGC-	PCR1= 415	
	AmpC-I-F-HDIII	-TCAAGCTTCAGGGCAGCCGCTTCGAC-		
	AmpC-R-BHI	-TCGGATCCCAGGTTGGCATCGACGAAG-	PCR2= 448	

[a]In the primer´s name; ERI, HDIII and BHI refer to the presence of the restriction sites (underlined sequence), EcoRI, HindIII and BamHI while F and R refer to the direction; forward and reverse respectively.

1. **Moya B, Juan C, Alberti S, Perez JL, Oliver A.** 2008. Benefit of having multiple ampD genes for acquiring beta-lactam resistance without losing fitness and virulence in Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy **52:**3694-3700.
2. **Moya B, Dotsch A, Juan C, Blazquez J, Zamorano L, Haussler S, Oliver A.** 2009. Beta-lactam resistance response triggered by inactivation of a nonessential penicillin-binding protein. PLoS pathogens **5:**e1000353.
3. **Quenee L, Lamotte D, Polack B.** 2005. Combined sacB-based negative selection and cre-lox antibiotic marker recycling for efficient gene deletion in pseudomonas aeruginosa. BioTechniques **38:**63-67.

Table S3. Relative abundance and retention time of muropeptides in peptidoglycan of the mutants and PAO1 wild type.

	Muropeptides (mol%)[a]								
	M3	M4G	M4	M5G	M5	D43	D45G	D44	D45
Strain	RT= 5.2	RT= 6.4	RT= 9.5	RT= 10.0	RT= 13.0	RT= 23.0	RT= 24.6	RT= 25.2	RT= 27.0
PAO1	10.5	1.1	24.9	0.3	0.3	6.2	ND	26.3	0.4
PAOΔ*dacB*	8.5(81)	0.9(82)	22.9(94)	0.4(133)	0.5(167)	5.3(85)	ND	28.9(110)	0.6(150)
PAOΔ*dacC*	10(95)	0.8(73)	25.4(102)	0.5(167)	1.4(467)	5.9(95)	ND	24.6(94)	2.8(700)
PAOΔ*pbpG*	8.9(85)	0.9(82)	25.2(101)	ND	0.6(200)	5.1(82)	ND	29(110)	0.2(50)
PAOΔ*dacB*Δ*dacC*	7.4(71)	0.7(64)	17.9(72)	1.4(467)	7.7(2567)	4.3(69)	2.5	17.3(66)	10.1(2525)
PAOΔ*dacB*Δ*pbpG*	6.7(64)	0.7(64)	20.3(82)	0.5(167)	0.6(200)	4.9(79)	ND	31.2(119)	ND
PAOΔ*dacC*Δ*pbpG*	8.7(83)	0.8(73)	23(92)	0.6(200)	1.7(567)	5.2(84)	1	25.8(98)	4.3(1075)
PAOΔ*dacB*Δ*dacC*Δ*pbpG*	3.4(32)	0.5(46)	6.4(26)	3.6(1200)	18.6(6200)	1.8(29)	4.2	8.5(32)	24.4(6100)

	Muropeptides (mol%)[a]					
	T444	T445	D44N	D45N	T444N	T445N
Strain	RT= 33.0	RT= 34.0	RT= 37.5-38	RT= 39.3	RT= 42.5	RT= 43.2
PAO1	2.3	ND	5	0.1	1.7	0.3
PAOΔ*dacB*	3.1(134)	ND	4.7(94)	0.1(100)	2.2(130)	0.3(100)
PAOΔ*dacC*	2.1(91)	0.3	4.8(96)	0.5(500)	1.7(100)	0.5(167)
PAOΔ*pbpG*	3(130)	ND	4.5(90)	0.1(100)	1.7(100)	0.4(121)
PAOΔ*dacB*Δ*dacC*	1.8(77)	1	3.3(66)	1.6(1600)	1.4(82)	1(333)
PAOΔ*dacB*Δ*pbpG*	4.3(187)	ND	4.5(90)	0.1(100)	2.3(135)	0.5(167)
PAOΔ*dacC*Δ*pbpG*	2.8(122)	0.5	4.4(88)	0.6(600)	1.7(100)	0.6(200)
PAOΔ*dacB*Δ*dacC*Δ*pbpG*	1.2(52)	3.3	1.1(22)	3.2(3200)	0.5(29)	2(667)

[a] Relative abundance in mol % of individual muropeptides. RT: retention time; ND: Not detected. Values in brackets represent percentage of the values obtained for each mutant related to wild-type PAO1.