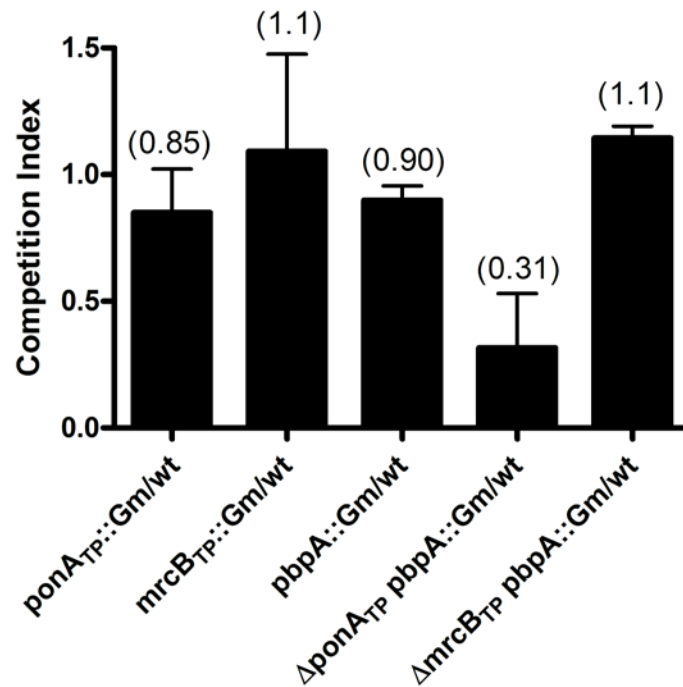


## Supplementary Data



**Fig. S1:** *In vitro* competition assay of PBP mutant strains. Cell cultures of each *pbp* replacement mutant (Gm<sup>r</sup>) was mixed with wild-type *Pa* (PA14) in LB broth flasks, incubated at 37°C for 7 h and spread on LB with or without gentamycin, as described in Experimental Procedures. The competition index (mutant/wild type) from three independent experiments was calculated and is shown in parentheses.

**Table S1: Primers used in this study**

Primers	Sequence	Purpose
PBP1A-UpF	GGCCAGTGCCAAGCTCTACAACCCGCTGGTCAAC	Knock out TP domain of PBP1a in PA14
PBP1A-UpR	TTTGAAGCTAATTCGAATCTGTACGAAGCGCAGG	
PBP1A-DnF	AAGATCCCCAATTCGATCAGGTACATGGGCTTCG	
PBP1A-DnR2	CCATGATTACGAATTCCGACTATCCGAACCAGGTC	
PBP1B-UpF2	GGCCAGTGCCAAGCTTGCTGCTGGAGCTGCACTAC	Knock out TP domain of PBP1b in PA14
PBP1B-UpR	TTTGAAGCTAATTCG GATCGGATCGAAGCTGGTG	
PBP1B-DnF	AAGATCCCCAATTCGACCGGTGCCTTGCAGGTG	
PBP1B-DnR2	CCATGATTACGAATTGGCACCATCACCACCACGC	
PBP2-UpF	GGCCAGTGCCAAGCTCTCGAACTGGTGGAGATCC	Knock out PBP2 in PA14
PBP2-UpR2	TTTGAAGCTAATTCGCTGCTTCATGCGCTTCTC	
PBP2-DnF2	AAGATCCCCAATTCGATGCAGCAGGTGGTGCATG	
PBP2-DnR	CCATGATTACGAATTGCATCAGCAGCTTCATGAAC	
PBP3-UpF2	GGCCAGTGCCAAGCTGGCTTCAGCTTCCTCAACG	Knock out PBP3 in PA14
PBP3-UpR	TTTGAAGCTAATTCGACGATTCGCCAGACGATG	
PBP3-DnF2	AAGATCCCCAATTCGCAGCAAGGTCATGGCTGGC	
PBP3-DnR2	CCATGATTACGAATTCAGCAGGTTGCTCAGGTTG	
pbp1A-GT-UPF	GGCCAGTGCCAAGCTAGTTCATCTCGGAAAGCC	Knock out GT domain of PBP1a in PA14
pbp1A-GT-UPR2	TTTGAAGCTAATTCGGGAGATCAGCTTGCCGTC	
pbp1A-GT-DnF3	AAGATCCCCAATTCGCAAGCTCGGCTTCATCGAC	
pbp1A-GT-DnR	CCATGATTACGAATTCTCTGCTCGAAGGAGAAGC	
pbp1B-GT-UPF	GGCCAGTGCCAAGCTGATCAGGGTCTGGCTCAC	Knock out GT domain of PBP1b in PA14
pbp1B-GT-UPR2	TTTGAAGCTAATTCGGATCAGCAGCGGCTCAAG	
pbp1B-GT-DnF3	AAGATCCCCAATTCGGACCCAGCAGGAAGTCGAC	
pbp1B-GT-DnR	CCATGATTACGAATTGCGACCTCCATCGGACTG	
pbp3x-UpF	GGCCAGTGCCAAGCTTAACTGCGCGAACTGATGGAC	Knock out PBP3x in PA14
pbp3x-UpR	TTTGAAGCTAATTCGTATGCAAGGGCACACTGG	
pbp3x-DnF	AAGATCCCCAATTCGGACAACCTGCAGGACAGCC	
pbp3x-DnR	CCATGATTACGAATTCCAGCAGGTGTAGTTCAGCAC	
SCN-pbp1A-F	TTGAGCCTGGCCGAGATG	Screening primers for PBP1a TP mutant
SCN-pbp1A-R	GCAACGACGTGATCATGG	
SCN-pbp1B-F	CCTGAGCAACGAACGCAG	Screening primers for PBP1b TP mutant
SCN-pbp1B-R	GGAAAGTCTGGATCGGTGC	
SCN-pbp2-F	GGAAGGCTGGCAGGAATAC	Screening primers for PBP2 mutant
SCN-pbp2-R	CGCTTGGACAGGTACCAG	
SCN-pbp3-F2	GAACTGGGCGATGAGGTG	Screening primers for PBP3 mutant
SCN-pbp3-R2	GTAGTCCACCACCACCAGC	
SCN-pbp1aTG-F	CACTTCCTCCAGCGGATAG	Screening primers for

SCN-pbp1aTG-R	GGTGAAGCCGTTGTCCAG	PBP1a GT mutant
SCN-pbp1bTG-F	GATGACCTCGAAGCGCTC	Screening primers For PBP1b GT mutant
SCN-pbp1bTG-R	AGTGAGGACGCTGCGGATC	
SCN-pbp3x-F	GGACCTCTGGAGCGGCTTC	Screening primers For PBP3x mutant
SCN-pbp3x-R	CGCTTCGTGATCGACATGG	
mini-CTX1-F	CCGTCCTTGCTGAATTAG	Flanking primers of pMini-CTX1
mini-CTX1-R	TCAAAAGGTCATCCACCG	
Pser-up	CGAGTGGTTTAAGGCAACG	Flanking primers of <i>attB</i> site in PA14
Pser-down	TCGGCCTGGTGGAAACAAC	
GFP-F	AAGCATATGGCCATGAGTAAAGGAGAAGAAC	Amplification of GFP
GFP-R	TTTGTATAGTTCATCCATGC	
GFP-PBP1a-F	AAGCATATGGCCATGGCGCGCCTGCTGAAGTTCC	pCAB-gfp-ponA
pbp1A-R	GAATTGGGTACCATGTCAGAACAGGTTCGATCGGC	
GFP-PBP3-F	GATGAACTATACAAAATGAACTGAATTATTTCC	pCAB-gfp-ftsI
PBP3-R	GAATTGGGTACCATGTCAGCCACGCCCTCCTTTTG	
7605-F	GCAGCCCGGGGGATCCG	For amplification of mCherry
mCherry-R2	CTTGTACAGCTCGTCCATGCC	
mCherry-PBP1A-F	GACGAGCTGTACAAGATGCGCCTGCTGAAGTTCC	For pMP7605-PBP1a
mCherry-PBP1A-R	TAGAACTAGTGGATC <sub>c</sub> TCAGAACAGGTTCGATCGGC	
mCherry-PBP1B-F	GACGAGCTGTACAAGATGACGCGTCCCGATCC	For pMP7605- PBP1b
mCherry-PBP1B-R	TAGAACTAGTGGATC <sub>c</sub> TCAATTCAGCCAGCCTCGTAC	
mCherry-PBP2-F	GACGAGCTGTACAAGATGCCGCAGCCGATCCAC	For pMP7605- PBP2
mCherry-PBP2-R	TAGAACTAGTGGATC <sub>c</sub> TTACTGTTCAAGGGCGGG	
mCherry-PBP3-F	GACGAGCTGTACAAGATGAACTGAATTATTTCC	For pMP7605- PBP3
mCherry-PBP3-R	TAGAACTAGTGGATC <sub>c</sub> TCAGCCACGCCCTCCTTTTG	