Table S1: Plasmids and *Streptomyces coelicolor* and *Escherichia coli* strains

Strain or plasmid	Genotype, characteristic(s) and/or use	Reference or source
***Streptomyces coelicolor* A3(2) strains**		
M145	$SCP1^{-}$ $SCP2^{-}$	(1)
E117	Δ*rpfA-E* (*rpf* null)	(2)
J3385	3×Δ*PASTA*	(3)
***Escherichia coli* strains**		
DH5α	Routine cloning	
ET12567(pUZ8002)	*dam dcm*; with transmobilizing plasmid pUZ8002	(4,5)
Rosetta 2	Protein overexpression	Novagen
Plasmids		
pET15b	Overexpression of His_6 tagged proteins	Novagen
pMC177	pET15b carrying *rpfA*	(6)
pMC200	pET15b carrying *rpfA*Δ*LysM*	This study
pMC178	pET15b carrying *rpfB*	(2)
pMC179	pET15b carrying *rpfC*	(2)
pMC181	pET15b carrying *rpfD*	(2)
pMC201	pET15b carrying *rpfD*Δ*LytM*Δ*LysM*	This study
pMC202	pET15b carrying *rpfD*Δ*LysM*	This study
pMC203	pET15b carrying *rpfD*Δ*LytM*	This study
pMC182	pET15b carrying *rpfE*	(2)

Table S2: Oligonucleotides used in this study

Primer Name	Sequence (5'- 3')[1]	Use
0974 PP 5'	CAGTACCATATGGCCGACGCGCGACCTGGGAC	Overexpression of RpfD
0974 PP 3'	CAGTACGGATCCTCAGATCCTGACGCCGCCGGC	Overexpression of RpfD
0974ΔlytM PP 3'	CATCATGGATCCCCGGGTGGTCCCCTGCCCGC	Overexpression of RpfDΔLytM
SCO0974ΔlysM rev	TGCTCTTGCTTCTGCTCTTTCAGTCCGGCCCGCTCCGAGCA	RpfDΔLysM overexpression
SCO0974ΔlysM fwd	AAAGAGCAGAAGCAAGAGCA	RpfDΔLysM overexpression
0974ΔlytMΔlysM PP3'	CATCATGGATCCTCCGGCCCGCTCCGAGCACA	Overexpression of RpfDΔLytMΔLysM
3097 PP 5'	CAGTACCATATGGCCACCGCGTCCG	Overexpression of RpfA
3097 PP 3'	CGAAGTGGATCCTTACTTCAGGTGCAGCTGCTG	Overexpression of RpfA
3097ΔlysM PP 3'	CATCATGGATCCTCAGCCGGTGCCGCA	Overexpression of RpfAΔLysM
T7 promoter	TAATACGACTCACTATAGGG	Sequencing
T7 terminator	GCTAGTTATTGCTCAGCGG	Sequencing

[1]Restriction enzyme sites are underlined

Table S3: Conditions for protein overexpression

	Induction OD_{600}	[IPTG] (mM)	Induction time (hr)	Induction temperature (˚C)	Molecular weight (kDa)[1]
RpfA	0.8	1	2.5	30	22.8
RpfAΔLysM	0.6	1	2.5	30	10.0
RpfB	0.8	0.25	16	16	36.6
RpfC	0.8	1	16	16	40.0
RpfD	0.8	1	4.5	30	42.6
RpfDΔLysM	0.8	1	16	16	30.3
RpfDΔLysMΔLytM	0.8	1	2.5	30	10.9
RpfE	0.8	1	2.5	30	10.2

[1] Calculated without the SignalP predicted signal peptide and with the 6×His tag

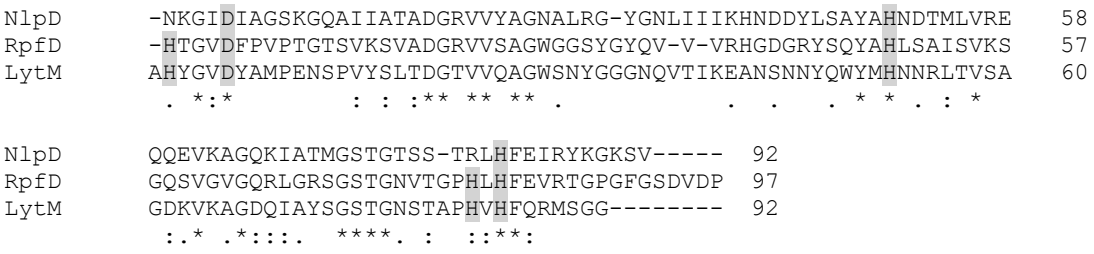

Figure S1: Catalytic residues in the LytM domain of *S. aureus* LytM are conserved in RpfD. Alignment of the amino acid sequence of LytM domains from *Escherichia coli* NlpD, *Staphylococcus aureus* LytM, and *Streptomyces coelicolor* RpfD, using Clustal Omega. LytM catalytic residues are highlighted in grey. Asterisks denote residues that are conserved, colons denote conservation of residues with strongly similar properties, and periods denote conservation of residues with weakly similar properties at a specific position.

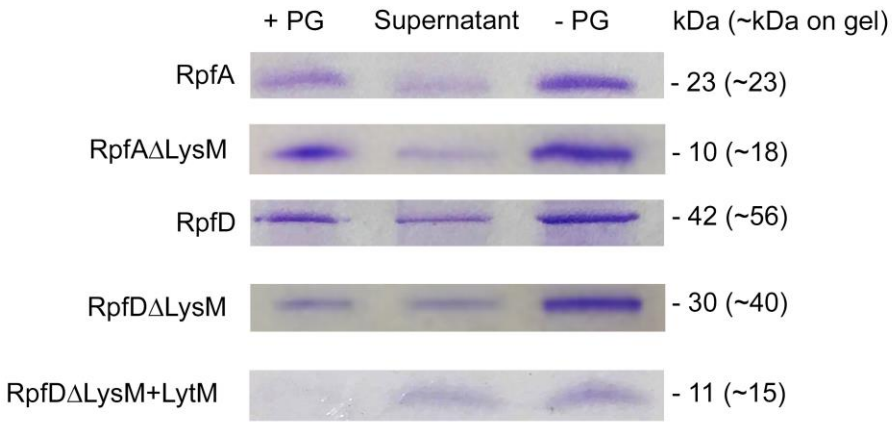

Figure S2: Peptidoglycan binding assays for RpfA and RpfD variants. Peptidoglycan binding by the different Rpf proteins was assessed by incubating approximately two nanomoles of each Rpf protein with peptidoglycan isolated from *Streptomyces coelicolor.* Following separation of bound (+PG, left) and unbound (Supernatant, centre) protein by centrifugation, the fractionated samples were separated on a tricine polyacrylamide gel (PAG) and stained using Coomassie Brilliant Blue. –PG (right) = input protein (positive control). Protein molecular weight is shown on the far right (kDa), with molecular weight relative to protein marker after separation on a PAG, shown in brackets (~kDa on gel).

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