

# RecA and specialized error-prone DNA polymerases are not required for mutagenesis and antibiotic resistance induced by fluoroquinolones in *Pseudomonas aeruginosa*

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## SUPPLEMENTARY MATERIAL

**Table S1.** Bacterial strains and plasmids used in this study.

Strain or plasmid	Genotype and/or relevant characteristics	Reference or source
<b><i>P. aeruginosa</i></b>		
PAO1 (ATCC15692)	Prototroph	American type culture collection
$\Delta recA$	PAO1 with an in-frame deletion of <i>recA</i> (PA3617)	[65]
$\Delta recA recA^+$	$\Delta recA$ with a copy of <i>recA</i> , including its own promoter, inserted into the <i>attB</i> site of the chromosome	This work
$\Delta polB$	PAO1 with an in-frame deletion of <i>polB</i> (PA1886)	[66]
$\Delta dinB$	PAO1 with an in-frame deletion of <i>dinB</i> (PA0923)	[66]
$\Delta imuC$	PAO1 with an in-frame deletion of <i>imuC</i> (PA0669)	[66]
$\Delta polB \Delta recA$	$\Delta polB$ deleted of <i>recA</i>	[66]
$\Delta dinB \Delta recA$	$\Delta dinB$ deleted of <i>recA</i>	[66]
$\Delta imuC \Delta recA$	$\Delta imuC$ deleted of <i>recA</i>	[66]
PAO1 CIP <sup>R</sup> -1	Ciprofloxacin resistant spontaneous mutant of PAO1, selected on agar plate supplemented with 1 µg/mL ciprofloxacin (8×MIC)	This work
PAO1 CIP <sup>R</sup> -1 $\Delta recA$	PAO1 CIP <sup>R</sup> -1 deleted of <i>recA</i>	This work
PAO1 CIP <sup>R</sup> -2	Ciprofloxacin resistant spontaneous mutant of PAO1, selected on agar plate supplemented with 1 µg/mL ciprofloxacin (8×MIC)	This work
PAO1 CIP <sup>R</sup> -2 $\Delta recA$	PAO1 CIP <sup>R</sup> -2 deleted of <i>recA</i>	This work
<b><i>E. coli</i></b>		
S17.1 $\lambda$ pir	<i>thi pro hsdRhsdM<sup>+</sup> recA RP4-2-Tc::Mu-Km::Tn7 <math>\lambda</math>pir</i> , Sm <sup>R</sup>	[67]
<b>Plasmid</b>		
pBluescript II KS+	Cloning vector; ColE1 replicon; Ap <sup>R</sup>	Stratagene
pDM4 $\Delta recA$	<i>sacB</i> -based suicide construct for the in-frame deletion of <i>recA</i> by homologous recombination	[65]
pDM4 $\Delta polA$	<i>sacB</i> -based suicide construct for the in-frame deletion of <i>polA</i> (PA5493) by homologous recombination	[66]
pDM4 $\Delta polB$	<i>sacB</i> -based suicide construct for the in-frame deletion of <i>polB</i> by homologous recombination	[66]
pDM4 $\Delta dinB$	<i>sacB</i> -based suicide construct for the in-frame deletion of <i>dinB</i> by homologous recombination	[66]
pDM4 $\Delta imuC$	<i>sacB</i> -based suicide construct for the in-frame deletion of <i>imuC</i> by homologous recombination	[66]
pDM4 $\Delta rmsA$	<i>sacB</i> -based suicide construct for the in-frame deletion of <i>rsmA</i> by homologous recombination	[38]

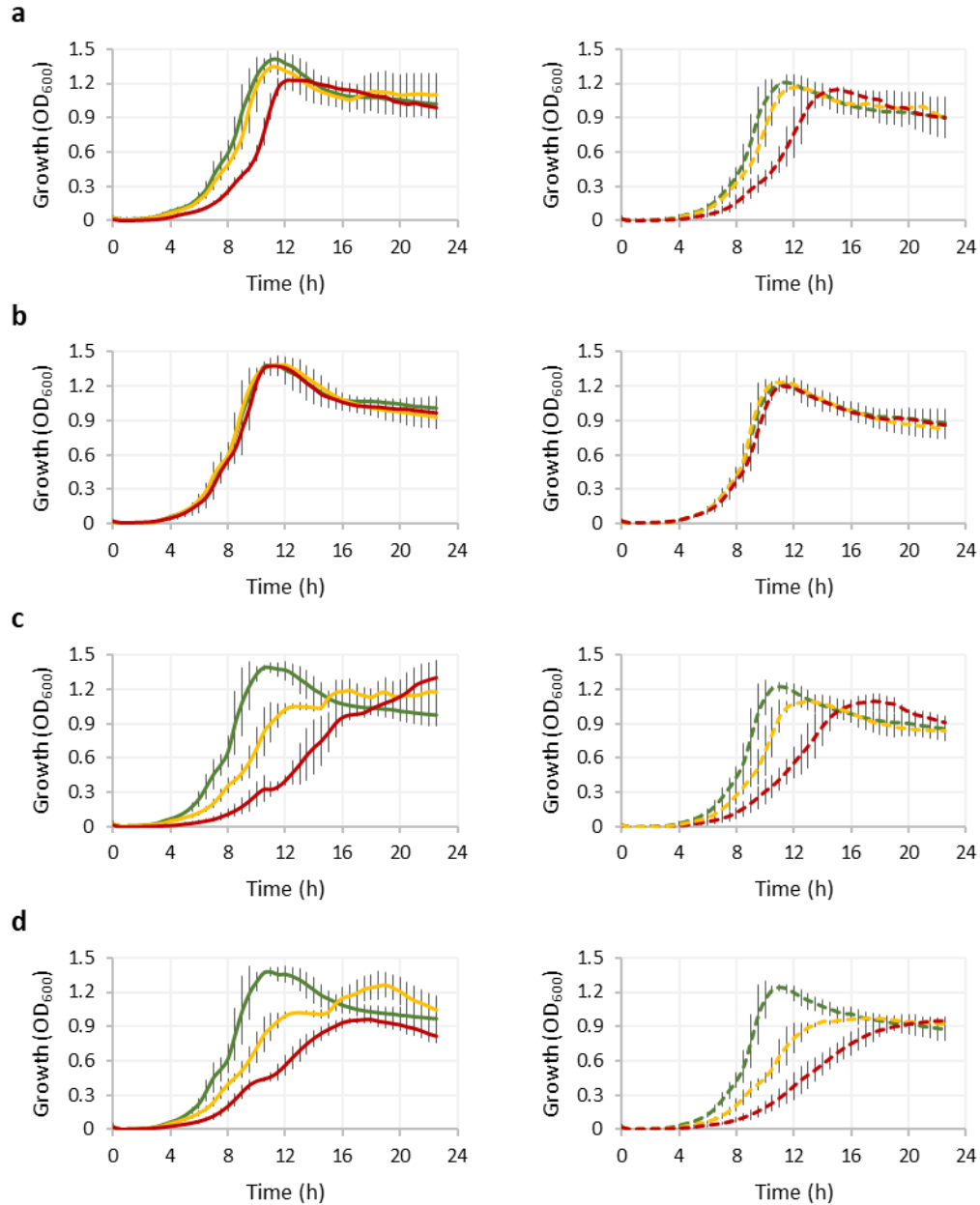
mini-CTX1	Self-proficient integration vector, $\Omega$ -FRT-attP-MCS, <i>ori</i> , <i>int</i> , <i>oriT</i> ; Tc <sup>R</sup>	[37]
mini-CTX1 <i>recA</i>	mini-CTX1 derivative carrying the <i>P. aeruginosa recA</i> gene with its own promoter	This study
pFLP2	Broad-host-range plasmid expressing the Flp recombinase, <i>sacB</i> ; Ap <sup>R</sup> /Cb <sup>R</sup>	[36]

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**Table S2.** Primers used in this study.

Primer name	Sequence (5'→3') <sup>a</sup>	Restriction site	Application
<i>recA</i> _mini-CTX1_FW	cgcaagc <u>TT</u> CGGAACATTCTTCCCGTC	HindIII	Generation of mini-CTX1 <i>recA</i>
<i>recA</i> _mini-CTX1_RV	ccggatCCTCAATCGGCTTCGGCG	BamHI	
<i>recN</i> _RT_FW	AGGAGCAGAAGACCCTGAGC		qRT-PCR
<i>recN</i> _RT_RV	GACAACAGGTTGACCGCTTC		
<i>recX</i> _RT_FW	CCGCTATCTCGAAAGCTTCA		
<i>recX</i> _RT_RV	CGCACTCCAGTCGACCTC		
<i>lexA</i> _RT_FW	GGCATGAGCATGAAGGACAT		
<i>lexA</i> _RT_RV	GAGCGAACTCAGGGTTTTCC		
<i>imuB</i> _RT_FW	GCCATGCTCTTCGAACTGAC		
<i>imuB</i> _RT_RV	TGTTCCCAGCCGAGATACTG		
<i>dinB</i> _RT_FW	GACTGTTTCTATGCCGCCCT		
<i>dinB</i> _RT_RV	CAGATCGGGACACAGCTTGA		
<i>polA</i> _RT_FW	TCGATTTCTCGCCCTGATG		
<i>polA</i> _RT_RV	TCGCGATTTTCTCGAGCTT		
<i>polB</i> _RT_FW	CTCGACTTCCTCCTCGAACG		
<i>polB</i> _RT_RV	CTTGTAGTCGAGCACCAGCA		
<i>polC</i> _RT_FW	GGTTATCGCAACCTCACCGA		
<i>polC</i> _RT_RV	GGAACACCTCCATCCACTCG		
<i>rpoD</i> _RT_FW	GGGCGAAGAAGGAAATGGTC		
<i>rpoD</i> _RT_RV	CAGGTGGCGTAGGTGGAGAA		
M13FW	GTTTTCCCAGTCACGAC		Sequencing of
M13RV	CAGGAAACAGCTATGAC		pBS constructs

<sup>a</sup> The restriction site used for cloning is underlined in the primer sequence.



**Figure S1.** Growth curves of *P. aeruginosa* PAO1 (left panels, solid lines) and the  $\Delta recA$  mutant (right panels, dotted lines) cultured in MH at 37°C in microtiter plates in the absence (green lines) or in the presence of (a) gentamicin, (b) colistin, (c) ofloxacin or (d) ciprofloxacin at 0.125×MIC (orange lines) or 0.25×MIC (red lines). Values are the mean ( $\pm$  SD) of three independent experiments.