Supplementary Materials

Target-Based Resistance in *Pseudomonas aeruginosa* and *Escherichia coli* to NBTI 5463, a Novel Bacterial Type II Topoisomerase Inhibitor

Nayar et al.

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Construction of pMMB67::nfxB expression plasmid. PAO1 genomic DNA was employed to amplify the *nfxB* gene using the primers NfxB-F and NfxB-R (Table S1). The forward sequence included the *nfxB* ribosome binding site, which is necessary for expression from the tac promoter on the pMMB67 plasmid (1). The gene was amplified using Q5 polymerase (New England Biolabs NEB), and the amplicon and pMMB plasmid were subsequently digested with *EcoRI* and *BamHI* (NEB) for 60 minutes. After digestion and concentration with a Zymo DNA Clean and Concentrator kit, the amplicon and plasmid were ligated for 60 minutes at 16℃ with T4 DNA ligase (NEB). The ligated DNA was transformed into competent *E. coli* DH5α and selected on LB ampicillin (100 μg/ml) plates. The next day a single colony was selected and inoculated into 10 ml of LB ampicillin broth, and after overnight growth, plasmid was purified (QIAprep Spin Miniprep Kit). The DNA was sequenced to confirm the correct *nfxB* sequence. The three Pseudomonas strains (AZ392, 394 and 396) were electroporated with both pMMB67EH and pMMB67::*nfxB* using the procedure of Choi et al (2). During MIC determinations, NfxB expression was induced with 100 μM IPTG.

1. **Fürste JP, Pansegrau W, Frank R, Blöker H, Scholz P, Bagdasarian M, Lanka E**. 1986. Molecular cloning of the plasmid RP4 primase region in a multi-host-range tacP expression vector. Gene 48: 119-131.
2. **Choi K-Y, Kumar A, Schweizer, HP**. 2006. A 10-min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: Application for DNA fragment transfer between chromosomes and plasmid transformation. J. Microbiol. Methods. 64: 391–397.

Table S1.
Primer sequences

Primer name	Sequence	Primer details
Asp-Glu-F	CCATCCCCATGGTGAATCGGCGGTCTATGAC	Quik change primers to make the Asp82Glu mutation in *gyrA* of *E.coli*
Asp-Glu-F-r	GTCATAGACCGCCGATTCACCATGGGGATGG	Quik change primers to make the Asp82Glu mutation in *gyrA* of *E.coli*
Asp-Gly-F	GGTAAATACCATCCCCATGGTGGCTCGGCGGTCTATGACACGATC	Quik change primers to make the Asp82Gly mutation in *gyrA* of *E.coli*
Asp-Gly-F-r	GATCGTGTCATAGACCGCCGAGCCACCATGGGGATGGTATTTACC	Quik change primers to make the Asp82Gly mutation in *gyrA* of *E.coli*
EcogyrAF	GCGACCTTGCGAGAGAAATTAC	forward primer to amplify the first 500 bp of *E.coli gyrA*
EcogyrAR	CTACGGCGATACCGGAAGAAC	reverse primer to amplify the first 500 bp of *E.coli gyrA*
YfaLP1	CACCCCGTTGCTTCACCGCATCGTCACTGATAGTGCGTGTAGGCTGGAGCTGCTTCG	Forward primer used to insert kanamycin resistant gene in *yfaL*
YfaLP2	GCACGGTAATCAGAAGCAAGGGGCCTGGCTGGATAGCATATGAATATCCTCCTTA	Reverse primer used to insert kanamycin resistant gene in *yfaL*
YfaLF	CATCACTCCCTTTCTGCCAC	forward primer to confirm the insertion of kanamycin resistant gene in *yfaL*
YfaLR	GAACTCGCGCCGATAACCAG	reverse primer to confirm the insertion of kanamycin resistant gene in *yfaL*
ygiU-P1	TTAGGTTATAACTAAAGTAACAGGGAGGCGGGGGTTGTGTAGGCTGGAGCTGCTTCG	Forward primer used to insert kanamycin resistant gene in *ygiU*
ygiU-P2	ACCATTTCTCCCTGGTGGCAAACCGGACATTTCATACATATGAATATCCTCCTTA	Reverse primer used to insert kanamycin resistant gene in *ygiU*
ygiU -F	GGTCACTATCTCCGTACATC	forward outside primer to confirm the insertion of kanamycin resistant gene in *ygiU*
ygiU -R	CGTCCACGGAAGGTGTATGG	reverse outside primer to confirm the insertion of kanamycin resistant gene in *ygiU*
ygiU -In-F	CTTGTCAATGCCGGGCAAGTTC	forward inside primer to confirm the insertion of kanamycin resistant gene in *ygiU*
ygiU -In-R	CGATCAGTACGTCATGAATTAC	reverse inside primer to confirm the insertion of kanamycin resistant gene in *ygiU*
ECA-A For	GTATAGGTTTACCTCAAACTGCGC	forward primer to amplify *E.coli gyrA*
ECA-A Rev	CAAAAGCCCAGACTTTGCAGCCTGG	reverse primer to amplify *E.coli gyrA*
EcGyBFor	GGGTAAAATAACGGATTAACCC	forward primer to sequence the 5' end of *E.coli gyrB*
EcGyBJ	GAGTCCCCTTCCACCAGGTAC	reverse primer to sequence the 5' end of *E.coli gyrB*
EcGyBC	CGTGGTTGGCAAAATTATCG	forward primer to sequence the 3' end of *E.coli gyrB*
EcGyBRev2	CGATATTCGCCGCTTTCAGG	reverse primer to sequence the 3' end of *E.coli*

		gyrB
ECC-For	CGGCAGATAATGTAGTATCTCCGG	forward primer to amplify *E.coli parC*
ECC-Rev	GACGACTTAACGTTTCATCCGGCG	reverse primer to amplify *E.coli parC*
EcParEFor	GGTCTGCACCATCTCTGACG	forward primer to sequence the 5' end of *E.coli parE*
NfxB-F	GGAATTCGCCAGTTTTCTGCACAATGCGC	Forward primer to amplify *P. aeruginosa nfxB*
NfxB-R	CGGGATCCGGTCAGGAGCGAGCCGGATTGG	Reverse primer to amplify *P. aeruginosa nfxB*
EcParEH	CAGCGATCCCAGATATCTTCC	reverse primer to sequence the 5' end of *E.coli parE*
EcParEB	GGTAATTTCGCTGGTGATACTG	forward primer to sequence the 3' end of *E.coli parE*
EcParEK	GCCATGTCGCCTTTCTCTTGC	reverse primer to sequence the 3' end of *E.coli parE*
ECA-A	CGTTATACGGAAATCCGTCTGGCG	sequencing primers for *E.coli gyrA*
ECA-B	TTCCGTATCAGGTAAACAAAGCGC	sequencing primers for *E.coli gyrA*
ECA-C	CGTGGCAGCTGGGCAACGTTGCCG	sequencing primers for *E.coli gyrA*
ECA-D	GTGGCGGGAAAGGTAAATCTGCCG	sequencing primers for *E.coli gyrA*
ECA-E	TCTTCTGTCCGTGCGATGGGCTGC	sequencing primers for *E.coli gyrA*
ECA-F	TGGTTGGGTATTCCGCCACTGCGG	sequencing primers for *E.coli gyrA*
ECA-G	GCAGGTTGACGATCGGACGACCGC	sequencing primers for *E.coli gyrA*
ECA-H	GCAGTTTTTCGTGCTCAAGACCGG	sequencing primers for *E.coli gyrA*
ECA-I	AAGAAACCTGCAACTGGGTCTGGG	sequencing primers for *E.coli gyrA*
ECA-J	CCTACGGCGATACCGGAAGAACCG	sequencing primers for *E.coli gyrA*
EcGyBA	CTGGAGCTGGTTATCCAGCG	sequencing primers for *E.coli gyrB*
EcGyBB	CGTTGCAGTGGAACGATGGC	sequencing primers for *E.coli gyrB*
EcGyBD	GTACAACCCGGACAAACTGCG	sequencing primers for *E.coli gyrB*
EcGyBE	GTTTGATGTTCACACCAATGC	sequencing primers for *E.coli gyrB*
EcGyBF	GTTGTTCACCACGCTGATGG	sequencing primers for *E.coli gyrB*
EcGyBG	CGTCATCCTGTACAGAGACAG	sequencing primers for *E.coli gyrB*
EcGyBH	GGAGTTGAGGAACGACAACTC	sequencing primers for *E.coli gyrB*
EcGyBI	GGAAACGACCGCAATCAGGC	sequencing primers for *E.coli gyrB*
EcGyBJ	GAGTCCCCTTCCACCAGGTAC	sequencing primers for *E.coli gyrB*
EcGyBK	CACTGGCGTTGGTGTGCAG	sequencing primers for *E.coli gyrB*
EcGyBL	CTGCTCGAAGCTGGCTACC	sequencing primers for *E.coli gyrB*
EcGyBRev	CCTGATAAGCGTAGCGCATC	sequencing primers for *E.coli gyrB*
ECC-A	CGTTACACCGAATCCCGGTTGTCG	sequencing primers for *E.coli parC*
ECC-B	GCGTACTGGAGCAAATTGCTGCGC	sequencing primers for *E.coli parC*
ECC-C	CGCGGTTTGGCCTTACGGAAACCC	sequencing primers for *E.coli parC*
ECC-D	GCCATTGACCCGATTACGCTGCCG	sequencing primers for *E.coli parC*
ECC-E	TTTTCCGGTAAGGTGATCAAAGCC	sequencing primers for *E.coli parC*
ECC-F	CGAACGACGATCGTCACCGTAGGC	sequencing primers for *E.coli parC*
ECC-G	TACGATAGCTCTTTTCCAGATCGG	sequencing primers for *E.coli parC*
ECC-H	AATATCGGTCGCCATGCCGACGGC	sequencing primers for *E.coli parC*

EcParEA	GGTCAGGTTTATAACATCGCC	sequencing primers for *E.coli parE*
EcParEC	GAACCAGAACGTTCAGGC	sequencing primers for *E.coli parE*
EcParED	GCAAAATCTGTATCCTCGCG	sequencing primers for *E.coli parE*
EcParEE	GGAAATGAACCCGATGCAATTGC	sequencing primers for *E.coli parE*
EcParEF	GGTGAATATCCACCGGCATC	sequencing primers for *E.coli parE*
EcParEFor2	GCTGATGCCATTGAGGTACTC	sequencing primers for *E.coli parE*
EcParEG	CATGCGTCAGGCGTGAAACAG	sequencing primers for *E.coli parE*
EcParEI	GGCATGATCGCCTGATATTCG	sequencing primers for *E.coli parE*
EcParEJ	CCGAGATCAATACGGTAGAGC	sequencing primers for *E.coli parE*
EcParERev	CTGACCGGGCAATGTTCTTTCC	sequencing primers for *E.coli parE*

Table S2. Complementation of second step mutants with plasmid based NfxB expression to restore MexCD OprJ efflux pump suppression

Strain	Genotype	MIC (μg/ml) NBTI 5463	Ciprofloxacin	Levofloxacin
AZ392	*nfxB*(Δ490-493), GyrA D82E	256	1	2
AZ394	*nfxB*(Δ490-493), GyrA D82N	256	8	8
AZ396	*nfxB*(Δ490-493), GyrA D82G	256	4	4
AZ799	AZ392(pMMB67)[1]	256	1	2
AZ800	AZ392 (pMMB67::*nfxB*)[2]	16	0.125	0.25
AZ801	AZ394 (pMMB67)[1]	256	4	8
AZ802	AZ394 (pMMB67::*nfxB*)	16	0.25	0.5
AZ803	AZ396 (pMMB67)[1]	128	2	2
AZ804	AZ396 (pMMB67::*nfxB*)	16	0.25	0.5

[1]pMMB67 empty vector control

[2]pMMB67 with cloned *nfxB* behind the *tac* promoter. 100 μM IPTG induction