Supplementary Material

Revisiting activation of and the mechanism of resistance to the compound IQG-607 in *Mycobacterium tuberculosis*

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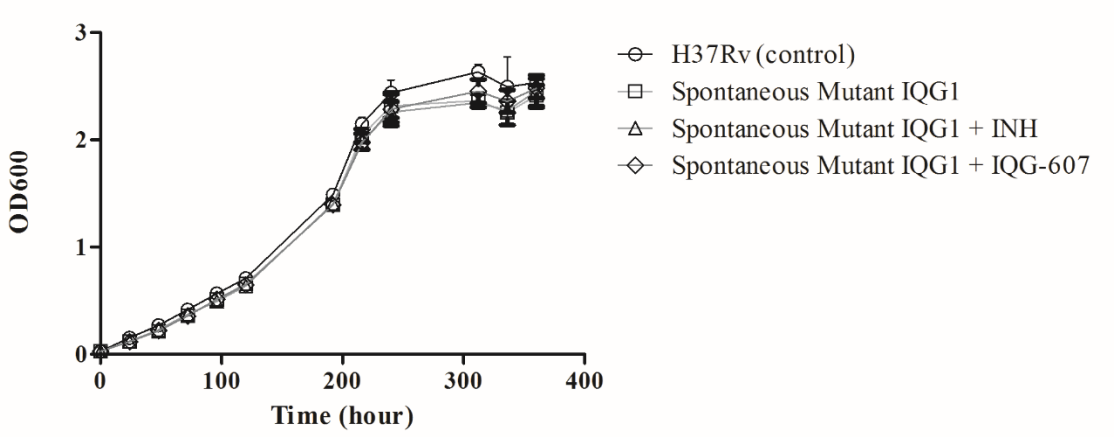
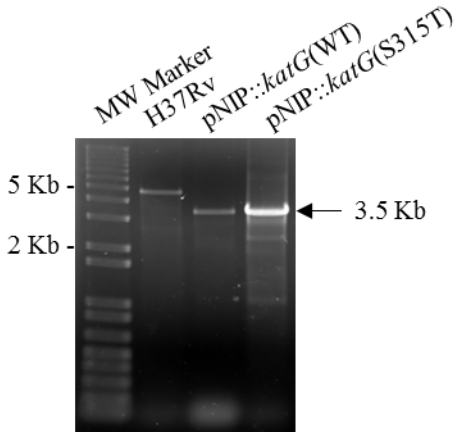
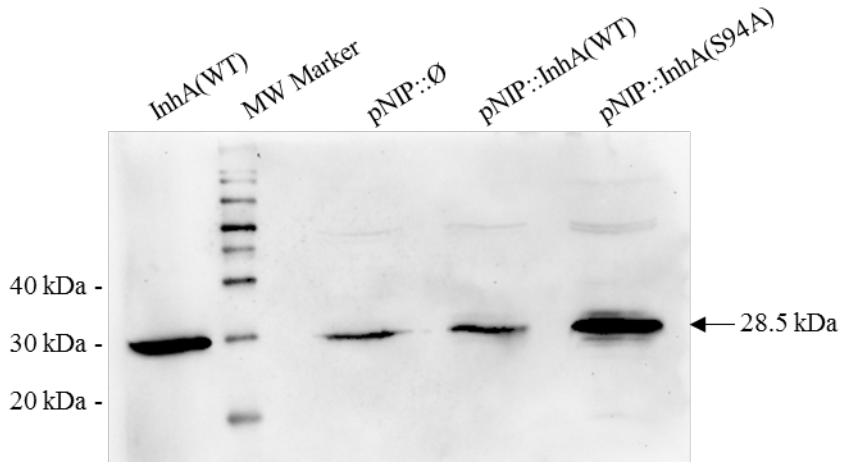
Contents:



Supplementary Fig. S1. Growth monitoring of the spontaneous mutant selected with IQG-607 (IQG1) in the absence (square) or in the presence of 16 mg/L of INH (triangle) or IQG-607 (diamond) over 15 days. H37Rv strain (circle) was used as a positive growth control of the experiment. The data were expressed as a mean value of triplicate optical density (OD_{600}) measurements. Two-way ANOVA with Bonferroni's post hoc test was employed to compare the growth of this mutant between the treatments.



Supplementary Fig. S2. Standard PCR confirming the disruption of the *katG* gene by the AES (allelic exchange substrate). The exchange of the WT *katG* by the AES, which contains the kanamycin gene resistance, reduces the original sequence size from 5.0 Kb to approximately 3.5 Kb. MW Marker = 1 Kb Plus DNA Ladder (Thermo Fisher).



Supplementary Fig. S3. Western Blot of cell extracts from *M. tuberculosis* strains overexpressing the InhA(WT) or InhA(S94A) enzymes. Each culture was grown until reach an OD_{600} between 0.8 and 1.0. Protein extraction and Western Blot assay were performed as previously described (1). Each well was loaded with 0.5 µg of protein extracts. The purified WT InhA protein (0.5 µg) with 28.5 kDa was obtained as described elsewhere (2), and was used as a positive control of the experiment. MW Maker = MagicMark XP Standard (Thermo Fisher). There was an increase of 1.5- and 3.7-fold in the InhA(WT) and InhA(S94A) expression, respectively, when compared to the underlying levels of the wild-type enzyme (pNIP::Ø).

1. Villela AD, Rodrigues VD, Pinto AF, Wink PL, Sánchez-Quitian ZA, Petersen GO, Campos MM, Basso LA, Santos DS. 2017. Characterisation of iunH gene knockout strain from *Mycobacterium tuberculosis*. Mem Inst Oswaldo Cruz 112:203–208.

2. Basso LA, Zheng R, Musser JM, Jacobs WR, Blanchard JS. 1998. Mechanisms of isoniazid resistance in *Mycobacterium tuberculosis*: enzymatic characterization of enoyl reductase mutants identified in isoniazid-resistant clinical isolates. J Infect Dis. 178:769-75.

Supplementary Table S1. Primers used in this study.

Name	Sequence	Purpose
KatG_F_SEQ	ATCGATGGGCTTCAAGACGT	Primers for *katG* sequencing
oligo_SEQ_R_001_katG	ATCAGCTTGTACCAGGCCTT	
SEQ_F_008_katG	GGAGGTCATCTACTGGGGTC	
SEQ_R_009_katG	ACGTAGATCAGCCCCATCTG	
SEQ_F_014_katG	CTGGAACACCCCGAGGAAT	
SEQ_R_015_katG	ATGTCGAGCAGGTTCACGAA	
PCR_R_013_KO_katG	AGACCAACCGTGTAGGCAAA	
SEQ_F_002_Prom_fabG-inhA	CTCGATGACGCAGATCTCGT	Primers for *inhA* promoter sequencing
SEQ_R_003_Prom_fabG-inhA	GTCACATTCGACGCCAAACA	
SEQ_F_004_ORF_inhA	TGCGAGCTATATCTCCGGTG	Primers for *inhA* ORF sequencing
SEQ_R_005_ORF_inhA	CAGGACGGCATCAAATTGCA	
PCR_inhA_WT_F_006	tttcatatgATGACAGGACTGCTGGACG	Primers for WT *inhA* PCR
PCR_inhA_WT_R_007	tttaagctttctagaCTAGAGCAATTGGGTGTGCG	
t280g_sense_MUT_S94A	CGACGGGGTGGTGCATGCGATTGGGTTCA	Primers for *inhA* site-directed mutagenesis
t280g_antisense_MUT_S94A	TGAACCCAATCGCATGCACCACCCCGTCG	
K_katG_UF	ttttGAATTCTGGTGGTCGGCAAG	Primers for PCR of the up/downstream *katG* sequences
K_katG_UR	ttttgcatgCGGCCGTCCGGAGCTGTTC	
K_katG_DF	ttttgcatgcGCCTGGGACAAGGTGATGAa	
K_katG_DR	ttttaagcttactagTGGGCTTGTTCGATCCCCATA	
PCR_F_010_KO_katG	GGACTGTACGTGCACTGGAT	Primers for *katG* knockout confirmation
PCR_R_011_KO_katG	ATGTCAATCCCCACCGCATA	
C_katG_F	tttttctagaGGACTGGCTGGGACACAA	Primer for *furA+katG* operon PCR
C_katG_R	tttttctagaAAATCGCGCCGGGCAAAC	
MSD_katG_F	CGGTAAGGACGCGATCACCACCGGCATCGAG GTCGTATGG	Primers for *katG* site-directed mutagenesis
MSD_katG_R	CCATACGACCTCGATGCCGGTGGTGATCGCG TCCTTACCG	

Supplementary Table S2. Evaluation of IQG-607 in combination with other drugs by the checkerboard assay.

Compound	∑FIC[a]	Outcome[b]
Rifampicin	2	Indifferent
Ethambutol	1	Indifferent
Moxifloxacin	1	Indifferent
Streptomycin	1	Indifferent
Pyrazinamide	1	Indifferent
D-cycloserine	2	Indifferent

[a]Fractional inhibitory index (∑FIC) were calculated based on the results from three independent experiments. [b]∑FICs of ≤0.5 suggest synergy and ≥4.0 indicates antagonism; values between suggest that both drugs act independently (indifferent). The highest concentration for each compound was: 10 mg/L for streptomycin, ethambutol and IQG-607; 50 mg/L for D-cycloserine and pyrazinamide; 0.3 mg/L for rifampicin and moxifloxacin.