

Supplementary Materials

Exploring the Molecular Mechanisms of Macrolide Resistance in Laboratory Mutant *Helicobacter pylori*

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Table S1. The combined bioinformatics analysis results of *H. pylori* G27 and Mutant-1 whole genome data.

CHROM	POS	REF	ALT	Codes For	Gene	aa change
NC_011333_G27	7125	GT	G	NA(non-coding region)	NA	NA
NC_011333_Mutant1	7125	GT	G	NA	NA	NA
NC_011333_Mutant1	20317	C	CTTTAATTAAG	NA	NA	NA
NC_011333_G27	74846	T	TA	urease accessory protein UreF CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	74846	T	TA	urease accessory protein UreF CDS	Pseudogene	Not a pseudogene
NC_011333_G27	156870	A	AT	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	156870	A	AT	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_G27	204054	T	TG	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	204054	T	TG	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	210816	G	GT	NA(non-coding region)	NA	NA
NC_011333_G27	232310	G	GA	NA(non-coding region)	NA	NA
NC_011333_Mutant1	232310	G	GA	NA(non-coding region)	NA	NA
NC_011333_G27	277049	A	AT	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	277049	A	AT	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_G27	277872	C	CA	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	277872	C	CA	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_G27	331419	G	GT	NA(non-coding region)	NA	NA
NC_011333_G27	334159	CT	C	tatA gene	Pseudogene	Not a pseudogene
NC_011333_Mutant1	334159	CT	C	tatA gene	Pseudogene	Not a pseudogene
NC_011333_Mutant1	355249	CAA	C	NA(non-coding region)	NA	NA
NC_011333_G27	401117	T	TG	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	401117	T	TG	hypothetical protein CDS	Pseudogene	Not a pseudogene
NC_011333_G27	431781	A	AT	helix-turn-helix transcriptional regulator CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	431781	A	AT	helix-turn-helix transcriptional regulator CDS	Pseudogene	Not a pseudogene
NC_011333_G27	433755	A	AG	outer membrane family protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	433755	A	AG	outer membrane family protein CDS	Pseudogene	Not a pseudogene
NC_011333_G27	441037	C	CT	pgsA gene	Pseudogene	Not a pseudogene
NC_011333_Mutant1	441037	C	CT	pgsA gene	Pseudogene	Not a pseudogene
NC_011333_G27	507732	C	CT	NA(non-coding region)	NA	NA
NC_011333_Mutant1	507732	C	CT	NA(non-coding region)	NA	NA
NC_011333_G27	508421	A	AC	NA(non-coding region)	NA	NA
NC_011333_Mutant1	508421	A	AC	NA(non-coding region)	NA	NA
NC_011333_G27	523568	G	GT	cag pathogenicity island protein CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	523568	G	GT	cag pathogenicity island protein CDS	Pseudogene	Not a pseudogene
NC_011333_G27	551897	G	GT	CagA		
NC_011333_Mutant1	551897	G	GT	CagA		
NC_011333_G27	555760	G	GA	rsmI gene	Pseudogene	Not a pseudogene
NC_011333_Mutant1	555760	G	GA	rsmI gene	Pseudogene	Not a pseudogene
NC_011333_G27	566958	A	AT	NA(non-coding region)	NA	NA
NC_011333_Mutant1	566958	A	AT	NA(non-coding region)	NA	NA
NC_011333_G27	581541	CT	C	energy transducer TonB CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	581541	CT	C	energy transducer TonB CDS	Pseudogene	Not a pseudogene
NC_011333_G27	581557	CT	C	energy transducer TonB CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	581557	CT	C	energy transducer TonB CDS	Pseudogene	Not a pseudogene
NC_011333_G27	581570	CT	C	energy transducer TonB CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	581570	CT	C	energy transducer TonB CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	657913	G	GC	NA(non-coding region)	NA	NA
NC_011333_Mutant1	694384	A	G	sulfite exporter TauE/Safe family protein CDS	sulfite exporter	L>P

CHROM	POS	REF	ALT	Codes For	Gene	aa change
NC_011333_G27	725944	T	TA	uvrA	Pseudogene	Not a pseudogene
NC_011333_Mutant1	725944	T	TA	uvrA	Pseudogene	Not a pseudogene
NC_011333_G27	739196	CG	C	DNA polymeras	Pseudogene	Not a pseudogene
NC_011333_Mutant1	739196	CG	C	DNA polymeras	Pseudogene	Not a pseudogene
NC_011333_Mutant1	748242	CAA	C	NA(non-coding)	NA	NA
NC_011333_Mutant1	758373	C	CA	NA(non-coding)	NA	NA
NC_011333_Mutant1	834623	CTT	C	NA(non-coding)	NA	NA
NC_011333_G27	936640	C	CA	NA(non-coding)	NA	NA
NC_011333_G27	1028566	A	G	hypothetical protein CDS		huge frameshift
NC_011333_G27	1208733	CA	C	hopl CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1208733	CA	C	hopl CDS	Pseudogene	Not a pseudogene
NC_011333_G27	1228406	CA	C	NA(non-coding)	NA	NA
NC_011333_Mutant1	1228406	CA	C	NA(non-coding)	NA	NA
NC_011333_G27	1228626	A	AT	NA(non-coding)	NA	NA
NC_011333_Mutant1	1228626	A	AT	NA(non-coding)	NA	NA
NC_011333_Mutant1	1242247	T	C	DUF874 family	primary mutation disrupt gene than secondary mutations make gene not becoming psuedogene	TNQTTHQPKHLPNSKQPSQR aa changes in final mutant
NC_011333_Mutant1	1242249	G	A			TNQTTTQPKHLPNSKQPHSQR aas in reference G27
NC_011333_Mutant1	1242264	G	T			
NC_011333_Mutant1	1242283	G	A			
NC_011333_Mutant1	1242287	T	G			
NC_011333_Mutant1	1251303	TAGGTTTTGGCGTCTTTTATCTTTGTGAGCCACTAATTGAGAGCTTAACTCAGGTTTTTTGGCGTCTTTTATCTTTGTGAGCCACTAATTGAGAGCTAACTCAGGTTTTTTGGCGTCTTTTATCTTTGTGAGCCACTAATTGAGAGCTAACTCAGGTTTTTTGGCGTCTTTTATCTTTGTGAGCCACTAATTGAGAGCTTA ACTCAGGTTTTTTGGCGTCTTTTATCTTTGTGAGCCACTAAT TGAGAGCTTAACTC	T	hypothetical pro	loss of a gene	
NC_011333_G27	1265037	C	CA	Name: DNA-dir	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1265037	C	CA	Name: DNA-dir	Pseudogene	Not a pseudogene
NC_011333_G27	1304662	A	AT	alaS CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1304662	A	AT	alaS CDS	Pseudogene	Not a pseudogene
NC_011333_G27	1305908	CT	C	babB CDS	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1305908	CT	C	babB CDS	Pseudogene	Not a pseudogene
NC_011333_G27	1333420	TA	T	NADH-quinone	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1333420	TA	T	NADH-quinone	Pseudogene	Not a pseudogene
NC_011333_G27	1333463	GC	G	NADH-quinone	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1333463	GC	G	NADH-quinone	Pseudogene	Not a pseudogene
NC_011333_G27	1333524	AT	A	NADH-quinone	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1333524	AT	A	NADH-quinone	Pseudogene	Not a pseudogene
NC_011333_G27	1359849	A	AT	DNA-directed R	frameshifted at terminal ends	secondary mutation corrects error
NC_011333_Mutant1	1359849	A	AT	DNA-directed R	frameshifted at terminal ends	secondary mutation corrects error
NC_011333_G27	1367919	GT	G	NA(non-coding)	NA	NA
NC_011333_Mutant1	1367919	GT	G	NA(non-coding)	NA	NA
NC_011333_Mutant1	1392764	C	CA	NA(non-coding)	NA	NA
NC_011333_G27	1410928	TA	T	4-hydroxybenzo	frameshifted at terminal ends	secondary mutation corrects error
NC_011333_Mutant1	1410928	TA	T	4-hydroxybenzo	frameshifted at terminal ends	secondary mutation corrects error
NC_011333_G27	1428286	G	GA	flhW CDS	frameshifted at terminal ends	secondary mutation corrects error
NC_011333_Mutant1	1428286	G	GA	flhW CDS	frameshifted at terminal ends	secondary mutation corrects error
NC_011333_G27	1480681	C	CT	mpa CDS	frameshifted at 5' end	secondary mutation corrects error
NC_011333_Mutant1	1480681	C	CT	mpa CDS	frameshifted at 5' end	secondary mutation corrects error
NC_011333_G27	1491244	T	TA	NA(non-coding)	NA	NA
NC_011333_Mutant1	1491244	T	TA	NA(non-coding)	NA	NA
NC_011333_G27	1491260	G	GA	NA(non-coding)	NA	NA
NC_011333_Mutant1	1491260	G	GA	NA(non-coding)	NA	NA
NC_011333_G27	1491283	GA	G	NA(non-coding)	NA	NA
NC_011333_Mutant1	1491283	GA	G	NA(non-coding)	NA	NA
NC_011333_G27	1541768	G	GA	heavy metal tra	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1541768	G	GA	heavy metal tra	Pseudogene	Not a pseudogene
NC_011333_G27	1568738	T	TA	outer membran	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1568738	T	TA	outer membran	Pseudogene	Not a pseudogene
NC_011333_Mutant1	1586573	CT	C	glycosyltransfer	in-silico SNP 6T repeat made Illumina sequencing error	NA
NC_011333_G27	1644852	AT	A	glycosyltransfer	in-silico SNP 6T repeat made Illumina sequencing error	

Table S2. The combined bioinformatics analysis results of *H. pylori* G27 and Mutant-2 whole genome data

Position of Polymorphism	Details of Polymorphism
1242247	/Reference_Nucleotide(s)="T" /Variant_Nucleotide(s)="C" /Change="T->C" /Polymorphism_Type="Substitution" /Qual="6569.77" /Failed_Filters="PASS" /Allele_count=2 /Allele_frequency=1 /Number_of_alleles=2 /Combined_Depth=150 /ExcessHet="3.0103" /FS="0.000" /MLEAC=2 /MLEAF=1 /Mapping_quality="42.39" /QD="44.09" /SOR="2.046" /Genotype="1/1" /AD="0,149" /Sample_Depth=149 /Genotype_Quality=99 /PL="6598,448,0" /label="C"
1242249	/Reference_Nucleotide(s)="G" /Variant_Nucleotide(s)="A" /Change="G->A" /Polymorphism_Type="Substitution" /Qual="6569.77" /Failed_Filters="PASS" /Allele_count=2 /Allele_frequency=1 /Number_of_alleles=2 /Combined_Depth=139 /ExcessHet="3.0103" /FS="0.000" /MLEAC=2 /MLEAF=1 /Mapping_quality="40.84" /QD="47.26" /SOR="2.186" /Genotype="1/1" /AD="0,139" /Sample_Depth=139 /Genotype_Quality=99 /PL="6598,448,0" /label="A"
1242264	/Reference_Nucleotide(s)="G" /Variant_Nucleotide(s)="T" /Change="G->T" /Polymorphism_Type="Substitution" /Qual="5731.77" /Failed_Filters="MQFilter" /Allele_count=2 /Allele_frequency=1 /Number_of_alleles=2 /Combined_Depth=116 /ExcessHet="3.0103" /FS="0.000" /MLEAC=2 /MLEAF=1 /Mapping_quality="38.00" /QD="49.41" /SOR="2.107" /Genotype="1/1" /AD="0,116" /Sample_Depth=116 /Genotype_Quality=99 /PL="5760,385,0" /label="T"

Table S2. The combined bioinformatics analysis results of *H. pylori* G27 and Mutant-2 whole genome data(continued)

Position of Polymorphism	Details of Polymorphism
1242283	/Reference_Nucleotide(s)="G" /Variant_Nucleotide(s)="A" /Change="G -> A" /Polymorphism_Type="Substitution" /Qual="6050.77" /Failed_Filters="MQFilter" /Allele_count=2 /Allele_frequency=1 /Number_of_alleles=2 /Combined_Depth=126 /ExcessHet="3.0103" /FS="0.000" /MLEAC=2 /MLEAF=1 /Mapping_quality="39.23" /QD="48.02" /SOR="1.953" /Genotype="1/1" /AD="0,126" /Sample_Depth=126 /Genotype_Quality=99 /PL="6079,412,0" /label="A"
1242287	/Reference_Nucleotide(s)="T" /Variant_Nucleotide(s)="G" /Change="T -> G" /Polymorphism_Type="Substitution" /Qual="5858.77" /Failed_Filters="PASS" /Allele_count=2 /Allele_frequency=1 /Number_of_alleles=2 /Combined_Depth=130 /ExcessHet="3.0103" /FS="0.000" /MLEAC=2 /MLEAF=1 /Mapping_quality="41.54" /QD="45.07" /SOR="2.452" /Genotype="1/1" /AD="0,130" /Sample_Depth=130 /Genotype_Quality=99 /PL="5887,406,0" /label="G"
1243410	/Reference_Nucleotide(s)="T" /Variant_Nucleotide(s)="C" /Change="T -> C" /Polymorphism_Type="Substitution" /Qual="2804.77" /Failed_Filters="PASS" /Allele_count=1 /Allele_frequency="0.5" /Number_of_alleles=2 /BaseQRankSum="9.370" /ClippingRankSum="0.000" /Combined_Depth=429 /ExcessHet="3.0103" /FS="2.013" /MLEAC=1 /MLEAF="0.5" /Mapping_quality="44.72" /MQRankSum="-0.662" /QD="6.54" /ReadPosRankSum="-2.656" /SOR="0.939" /Genotype="0/1" /AD="324,105" /Sample_Depth=429 /Genotype_Quality=99 /PL="2833,0,10407"

Table S2. The combined bioinformatics analysis results of *H. pylori* G27 and Mutant-2 whole genome data(continued)

Position of Polymorphism	Details of Polymorphism
1243461	/Reference_Nucleotide(s)="C" /Variant_Nucleotide(s)="T" /Change="C -> T" /Polymorphism_Type="Substitution" /Qual="5134.77" /Failed_Filters="PASS" /Allele_count=1 /Allele_frequency="0.5" /Number_of_alleles=2 /BaseQRankSum="-10.125" /ClippingRankSum="0.000" /Combined_Depth=693 /ExcessHet="3.0103" /FS="4.063" /MLEAC=1 /MLEAF="0.5" /Mapping_quality="52.33" /MQRankSum="-5.739" /QD="7.46" /ReadPosRankSum="2.685" /SOR="1.079" /Genotype="0/1" /AD="488,200" /Sample_Depth=688 /Genotype_Quality=99 /PL="5163,0,17376"
1243648	/Reference_Nucleotide(s)="G" /Variant_Nucleotide(s)="A" /Change="G -> A" /Polymorphism_Type="Substitution" /Qual="720.77" /Failed_Filters="MQRankSumLow;QDFilter" /Allele_count=1 /Allele_frequency="0.5" /Number_of_alleles=2 /BaseQRankSum="-6.455" /ClippingRankSum="0.000" /Combined_Depth=971 /ExcessHet="3.0103" /FS="47.951" /MLEAC=1 /MLEAF="0.5" /Mapping_quality="58.43" /MQRankSum="-29.608" /QD="0.74" /ReadPosRankSum="-2.724" /SOR="6.617" /Genotype="0/1" /AD="888,83" /Sample_Depth=971 /Genotype_Quality=99 /PL="749,0,37701"
1243650	/Reference_Nucleotide(s)="G" /Variant_Nucleotide(s)="A" /Change="G -> A" /Polymorphism_Type="Substitution" /Qual="720.77" /Failed_Filters="MQRankSumLow;QDFilter" /Allele_count=1 /Allele_frequency="0.5" /Number_of_alleles=2 /BaseQRankSum="3.239" /ClippingRankSum="0.000" /Combined_Depth=975 /ExcessHet="3.0103" /FS="47.769" /MLEAC=1 /MLEAF="0.5" /Mapping_quality="58.46" /MQRankSum="-29.838" /QD="0.74" /ReadPosRankSum="-3.160" /SOR="7.403" /Genotype="0/1" /AD="893,81" /Sample_Depth=974 /Genotype_Quality=99 /PL="749,0,37701"

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sequence1 - 23S rRNA   TAAGCCGAGTCCGAAATGGGAAGGTGATGGCAAATGGAAATATTCCAATACCGACTAT
NC_011333 - 23S rRNA 2 TAAGCCGAGTCCGAAAGGGGAGGTGATGGCAAATTGGTTAATATTCCAATACCGACTAT

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Figure S1. Position of SNPs detected (T1495A, T1494A, T1490A, T1476A, G1472T) via WGS in Mutant 1 strain.

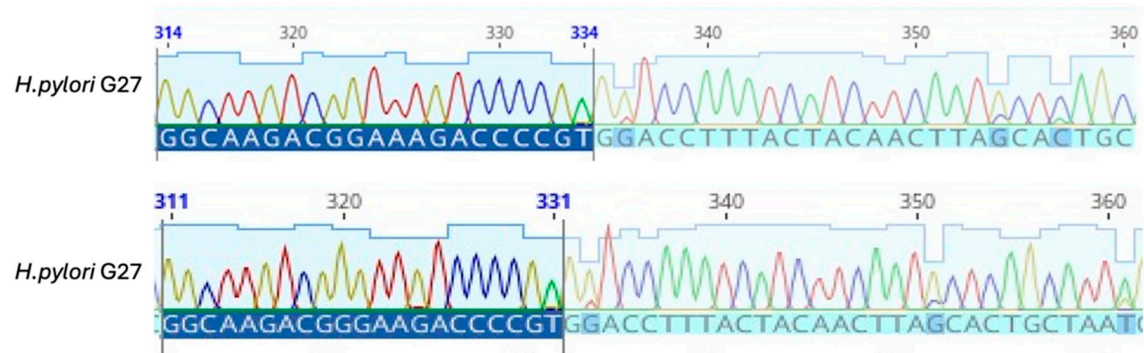


Figure S2: Signal decreases observed in Sanger sequence analysis are thought to be due to potential methylations occurring in the 23S rRNA region, leading to breaks.