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MODULATION OF *HELICOBACTER PYLORI* TRANSCRIPTIONAL PROFILE BY SUBINHIBITORY CONCENTRATIONS OF RIFAMPICIN

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Subinhibitory concentrations (*sub - MICs*) of antibiotics do not kill bacteria, but they are able to interfere with important aspects of bacterial cell function, such as adhesion to host cells, surface bacterial energy, susceptibility to host defense mechanisms, inhibition of enzyme function and toxin production. In order to understand how *H. pylori* copes with environmental stress and what facilitates the emergence of RIF mutants in *H. pylori,* we used DNA microarrays to compare the gene expression profiles of *H. pylori* in the presence and absence of subinhibitory concentrations of rifampicin (1/16 MIC (0.1 mg/L), 1/8 MIC (0,2mg/L), ¼ MIC (0,4 mg/L), and ½ MIC (0,8 mg/L). We found that ehe expression of 57 genes (of the 1,576 genes analyzed) was increased more than ≥ 1,5 – fold, and the expression of only 29 genes was decreased more than ≤ 1,5 –fold in significant way (p-value < 0,05), when *H. pylori* was treated with *sub – MICs* of RIF. No correlation was found between the *sub - MICs* of RIF and gene expression. We conclude that the alteration in the transcriptional pattern of *H. pylori* after the exposure to *sub – MICs* of RIF is mainly due to a direct interaction between rifampicin and the RNA polymerase β-subunit. Finally, we propose that subinhibitory concentrations of rifampicin may lead to an increase in the number of hypermutable cells in the *H. pylori* population.

Keywords: ***Helicobacter pylori*, selection, microarray, *rpoB.***

INTRODUCTION

In bacterial populations, an increased mutational frequency under conditions of environmental stress may confer a selective advantage. The presence of antibiotics, even at low concentrations, may constitute an environmental stress. Subinhibitory concentrations (*sub - MICs*) of antibiotics do not kill bacteria, but they are able to interfere with important aspects of bacterial cell function, such as adhesion to host cells, surface bacterial energy, susceptibility to host defense mechanisms, inhibition of enzyme function and toxin production (reviewed in 10). An increase in the mutation frequency has been observed in different bacterial populations after exposure to *sub - MIC* antibiotics [13, 16, 30]. For *S. pneumonia*, subinhibitory levels of ciprofloxacin and streptomycin increase the frequency of acquiring rifampicin resistance between 2-and 5-fold, which is comparable to the increase seen in mismatch repair mutants of this species [16]. When *M. fortuitum* is exposed to $^{1}/_{2}$ MIC of ciprofloxacin, its mutation rate increases by 72- to 120-fold during a selection with quinolones or other antimycobacterial antibiotics [13]. Smaller but significant increases in the mutation rate were also seen when the microorganism was exposed to lower concentrations of ciprofloxacin ($^{1}/_{4}$ MIC and $^{1}/_{8}$ MIC).

The stress response mechanisms that cause an increase in mutation frequency have been widely studied. Antibiotic treatments induce the SOS response, which upregulates multiple genes involved in DNA repair, recombination, mutation, and other functions [8, 9, 17, 21]. The SOS response is controlled by LexA, a transcriptional repressor. DNA damage triggers LexA autocleavage, which derepresses the SOS genes controlled by LexA. Once activated, the SOS response promotes the increase in mutational frequency largely through the induction of error-prone DNA polymerases [2, 17]. Recent studies have shown that the activation of error-prone polymerases occurs at the end of stationary phase and during starvation [18].

The same stress response mechanisms that allow *E.coli* to adapt to a broad range of stressful conditions are not present in some bacteria from other taxa. Unlike many other bacterial organisms, *H. pylori* does not have a LexA ortholog [1, 24]; it also lacks many genes involved in DNA repair, recombination, and mutagenesis, such as the *mutHL* genes (methyl-directed mismatch repair), the *umuCD* genes (UV-induced mutagenesis), and the SOS-controlled error-prone DNA polymerases. These observations suggest that *H. pylori* may not have a typical SOS response. Nonetheless, ~1/4 of the *H. pylori* strains isolated from dyspeptic patients show higher mutation frequencies to rifampicin (RIF) resistance than *Enterobacteriaceae* mismatch-repair defective mutants [5]. The

exposure of *H. pylori* to *sub - MICs* of RIF can help us to understand how *H. pylori* copes with environmental stress and what facilitates the emergence of RIF mutants in *H. pylori.*

Here, we examined the gene expression profiles of *H. pylori* in response to treatments with different *sub - MICs* of RIF. Multiple concentrations of antibiotics were applied to examine dose-specific effects.

MATERIALS AND METHODS

H. pylori strains and growth medium. H. pylori reference strain 26695 was routinely maintained on Columbia agar (bioMerieux, France) plates supplemented with 10% heat-inactivated Horse Serum (PAA Lab., Austria), 5% yeast extract (Russia), and *H. pylori*-selective antibiotic mix Dent (Oxoid). Liquid culture was grown in BHI broth (3,7% brain heart infusion (bioMerieux, France) with 10% inactivated Fetal Bovine Serum (HyClone, USA) and 5% yeast extract). Both plates and broth cultures were incubated at 37° under an atmosphere enriched with 5% CO_2 for 2-3 days.

DNA extraction and sequencing of rpoB. Fresh cultures (middle log phase) of *H. pylori* were suspended in BHI broth (~ 10^5 cells/ml) with 10% inactivated Fetal Bovine Serum, to which RIF had been added at concentrations equivalent to 1/16 MIC (0,1 mg/L), 1/8 MIC (0,2mg/L), ¼ MIC (0,4 mg/L), and ½ MIC (0,8 mg/L) and were incubated at 37° under an atmosphere enriched with 5% CO_2 for 24 hour. Then, chromosomal DNA from wild and sub-MIC treated *H. pylori* strains was isolated with Wizard Genomic DNA Purification (Promega). The PCR reactions were performed under the following conditions: 94°C 3 min, followed by 30 cycles of 94°C 10 s, 55°C 10 s, 72°C 30 s, with a final elongation at 72°C for 5 min. DNA sequencing was carried out on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using ABI Prism BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit according to the manufacturer's recommendations; sequencing was done in both directions using the same primers as those used for amplification (Table 1).

Table 1. PCR primers for sequencing

Primers	Sequence (5' → 3')	Gene region	Fragment size	Position and direction
rpoB1 rpoB2	tttgattcgctcatgccccat cacaaccttttataaggggc	Cluster I + cluster II	335 bp	bp 1492, sense bp 1827, antisense
rpoB149f rpoB149r	gatccctttgatgacagaacgc tccctaccataacaggctcagc	V149	532 bp	bp 387, sense bp 919, antisense
rpoB2f rpoB701r	gccccttataaaaaggttgtg gcgcacatttttccctaacg	R701	626 bp	bp 1807, sense bp 2433, antisense

RNA isolation. Total RNA from *H. pylori* strains incubated at different *sub - MICs* of RIF was extracted using a commercial RNAqueous-4PCR purification kit (Ambion, USA). The quantity and the quality of the extracted RNA were checked by microcapillary electrophoresis using a Bioanalyzer 2100 (Agilent Technologies, Wilmington, DE, 4 USA).

Microarray analysis. The primer set for PCR was purchased from Eurogentec. A total of 3242 primers were designed and synthesized to amplify complete ORFs in the *H. pylori* genome of strain 26695. The primers were designed to amplify each ORF beginning at the start codon and ending at the stop codon. The PCR reactions were performed in 96-well plates in a 100 µl reaction volume in an MJ Research (Cambridge, MA) PTC-225 DNA Engine Tetrad thermal cycler; the following conditions were used: 94°C 5 min; followed by 30 rounds of 30 seconds at 94°C, 30 seconds at 50°C, and 4 min at 72°C; followed by one round of 10 min at 72°C. To assay the yield and specificity of the amplified ORFs, all PCR reactions were analyzed by electrophoresis on a 1% agarose gel. The PCR products of 1576 ORFs were successfully obtained (97%).

The DNA chips were manufactured at the Biochip platform of Toulouse – Genopole on UltraGAPS slides (Corning) using PCR products. Fluorescent-labeled cDNA was synthesized from 5 µg of total RNA using the ChipShot Direct Labeling System (Promega). Labeled cDNA was purified using the ChipShot Membrane Clean-up Systems (Promega). Hybridization was carried out in an automatic hybridization chamber (Discovery™, Ventana). Microarrays were prehybridized in a solution of 1% BSA, 2 x SCC, and 0,2% SDS for 30 min at 42ºC followed by the addition of a mixture containing 200 µl of ChipHybe™ (Ventana), 10 µl of Cy3-labelled cDNA, and 10 µl of Cy5-labelled cDNA. After 14 hr of hybridization at 42ºC, the DNA chips were washed for 5 min in 2 x SSC, 0,1% SDS at room temperature and 2 times for 2 min each in 0,1 x SSC buffer at room temperature. The hybridization signal was detected with a GenePix 4000B laser Scanner (Axon Instruments), and the signal quantification was transformed to numerical values using the integrated GenePix software version 3.01. Experiments (from RNA extraction to image analysis) were repeated two times by swapping the fluorescent dyes CY3 and CY5 to reduce the number of false positives due to dye effects.

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Data acquisition and analysis. All raw data and full details of normalization and statistical regimes performed using Bioplot software developped at platform Biochips and presented at http://biopuce.insa-toulouse.fr/. This software is an online web service available to all users of the Biochips platform. A complete user's guide is available at http://biopuce.insa-toulouse.fr/ExperimentExplorer/doc/BioPlot/. Raw intensities were background-corrected, log transformed, and normalized by the mean log-intensity of all spots. Log-ratios of normalized intensities from duplicate samples were tested for statistical significance using Student's paired bi-tailed *t*-test. To reduce the false discovery rate, we tested only genes with at least a 1,5-fold variation, and the p-value threshold in the Student's *t*-test was set at ≤ 0,05. To determine the degree to which transcription of a particular gene was regulated at a given concentration of RIF, the normalized value from that concentration was divided by the corresponding value from the wild type and converted to $\log_{10}$. Positive and negative values defined, respectively, up and down-regulated genes under the studied conditions.

Real-time qPCR. Real-time PCR was used to verify the data obtained using the arrays. The reverse transcription reaction was carried out as follows. RNA (1 µg) was first denatured at 94°C for 30 sec in 10 µl of a mixture containing (besides RNA) four types of NTPs (0,5 mM each) and 150 pmol of random hexamer primers. For primer annealing, the mixture was incubated on ice for 30 min. Then, 20 U RNase inhibitor (Promega, USA), 200 U MMLV reverse transcriptase (Promega), and buffer for reverse transcription were added, and the resultant mixture was incubated at 37°C for 30 min. PCR was carried out in a volume of 50 µl containing 10 pmol of primers (Table 2), SybrGreen 0.003%, 25 mM Tris-HCl, pH 8.3, 2,5 mM MgCl2, 50 mM KCl, a mixture of four types of dNTPs (0.2 mM each), and 2 U Taq-polymerase. Products were amplified and detected using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, USA) and MicroAmp Optical 96-well reaction plate/optical caps (Applied Biosystems) in the following mode: denaturation at 94°C for 2 min, followed by 40 cycles: 93°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec. Amplification results were assessed using the Sequence Detection System software version1,6 (Applied Biosystems).

Table 2. *H. pylori* genes whose expression is induced or repressed in response to subinhibitory concentrations of rifampicin

Expression	Functional Group - Main category	1/16 MIC	1/8 MIC	1/4 MIC	1/2 MIC	ORF ID	Gene name
Induced	Biosynthesis of cofactors, prosthetic groups, and carriers	1.10	1.45	1.60	0.83	HP0034	aspartate 1-decarboxylase (*panD*)
		1.38	1.46	1.53	1.36	HP0293	para-aminobenzoate synthetase (*pabB*)
		0.73	1.17	1.14	1.57	HP1036	7. 8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (*folK*)
	Cellular processes	0.94	1.31	1.20	1.51	HP0327	flagellar protein G (*flaG*)
		0.54	1.04	1.11	1.75	HP0536	Cag pathogenicity island protein (*cag15*)
	Central intermediary metabolism	1.21	1.51	1.55	1.18	HP0071	urease accessory protein (*ureI*)
		1.19	1.45	1.55	1.05	HP1186	carbonic anhydrase
	DNA metabolism	0.93	1.18	1.24	1.68	HP0091	type II restriction enzyme R protein (*hsdR*)
		1.04	1.95	1.34	1.37	HP0602	endonuclease III
		1.10	1.36	1.53	1.08	HP0925	recombinational DNA repair protein (*recR*)
	Energy metabolism	1.37	1.54	1.59	1.32	HP1131	ATP synthase F1, subunit epsilon (*atpC*)
	Fatty acid and phospholipid metabolism	1.41	2.03	1.68	1.56	HP0559	acyl carrier protein *(acpP)*
		1.36	1.46	1.53	1.14	HP1016	phosphatidylglycerophosphate synthase (pgsA)
	Hypothetical	1.08	1.41	1.64	1.24	HP0234	conserved hypothetical integral membrane protein
		1.39	1.41	1.65	1.49	HP0759	conserved hypothetical integral membrane protein
		1.25	1.42	1.59	1.27	HP1423	conserved hypothetical protein
		0.84	1.38	1.29	1.50	HP1587	conserved hypothetical protein
	Other Categories	1.36	1.71	1.81	1.46	HP1008	IS200 insertion sequence from SARA17
	Pyrimidine ribonucleotide biosynthesis	1.36	1.49	1.60	1.36	HP0266	dihydroorotase (*pyrC*)
	Transcription	1.01	1.24	1.30	1.56	HP0662	ribonuclease III (*rnc*) {Escherichia coli}

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		1.36	1.59	1.42	1.27	HP1198	DNA-directed RNA polymerase. beta subunit (*rpoB*)
	Translation	1.05	1.55	1.81	1.66	HP1040	ribosomal protein S15 (*rps15*)
	Transport and binding proteins	1.10	1.53	1.36	1.08	HP0888	iron(III) dicitrate ABC transporter. ATP-binding protein (*fecE*)
		1.32	1.59	1.54	1.37	HP1169	glutamine ABC transporter. permease protein (*glnP*)
Repressed	Amino acid biosynthesis	0.75	0.59	0.74	0.78	HP0107	cysteine synthetase (*cysK*)
	Fatty acid and phospholipid metabolism	0.86	0.86	0.78	0.61	HP0499	phospholipase A1 precursor (DR-phospholipase A)
		0.57	0.69	0.64	0.76	HP0644	conserved hypothetical integral membrane protein
	Central intermediary metabolism	0.82	0.83	0.88	0.59	HP0047	hydrogenase expression/formation protein (*hypE*)
	Hypothetical	1.13	0.76	0.90	0.65	HP0035	conserved hypothetical protein
	Protein fate	0.56	0.56	0.61	0.34	HP0011	co-chaperone (*groES*)
	Regulatory functions	0.99	0.94	0.99	1.53	HP0166	response regulator (*ompR*)
	Translation	0.95	0.93	0.79	0.61	HP0827	ss-DNA binding protein 12RNP2 precursor
	Transport and binding proteins	0.91	0.80	0.81	0.65	HP0607	acriflavine resistance protein (*acrB*)
		0.74	0.65	0.77	0.63	HP1427	histidine-rich, metal binding polypeptide (*hpn*)

RESULTS

To understand the adaptive response of *H. pylori* to *sub - MICs* of RIF, a DNA microarray technique was used to analyze the transcriptional changes in *H. pylori* after exposure to different *sub - MICs* of RIF. It should be noted, that the pre-incubation of *H. pylori* with *sub-MICs* of RIF does not lead to the emergence of mutations in the *rpoB* gene that confer resistance.

Microarrays containing 1,576 ORFs of *H. pylori* strain 26695 were hybridized with fluorescently labeled cDNAs synthetised from total RNA extracted from *H. pylori* cultures that had been incubated either in the control condition or in various *sub – MICs* of RIF. A ratio of intensity of the Cy5 and Cy3 fluorophore at each spot was obtained by scanning with an Axon scanner; this ratio represents the change in total mRNA content after exposure *sub – MICs* of RIF. To reduce the number of false positive values due to dye effects, experiments (from RNA extraction to image analysis) were repeated two times, swapping the fluorescent dyes Cy3 and Cy5.

The expression of only 57 genes (of the 1,576 genes analyzed) was increased more than ≥ 1.5 – fold, and the expression of only 29 genes was decreased more than ≤ 1.5 –fold in significant way (p-value < 0,05), when *H. pylori* was treated with *sub – MICs* of RIF. Hence, the expression of the vast majority of the genes analyzed was not detectable during the exposure to *sub – MICs* of RIF. The average change in the 57 up-regulated genes and 29 down-regulated genes is shown in Tables 2 and 3. The expression level of only gene, HP0412, increased by more than twofold. The expression of the other genes changed by less than twofold. When the 24 up-regulated genes with known function were classified by function, several different types of genes were found and are shown in Table 2. The *rpoB* gene, encoding the beta subunit of the DNA-directed RNA polymerase, a target of RIF, was one of those identified. Also, genes involved in biosynthesis/ metabolism processes (*panD, pabB, folK, atpC, acpP, pgsA, pyrC,* HP1186), DNA metabolism (*hsdR, HP0602, recR*), transcription/translation (*rnc, rps15*), transport (*fecE, glnP*), and cellular processes (*flaG, cag15*) were upregulated. Genes that were down-regulated upon exposure to *sub – MICs* of RIF belong to the same functional groups, except for *groES,* which encodes a co-chaperone (protein fate), and *ompR*, which encodes a response regulator (regulatory functions). Three differentially expressed genes, *hypE* (HP0047), carbonic anhydrase (HP1186), and *ureI* (HP0071), are pH regulated genes.

To identify patterns of gene expression that are regulated by *sub – MICs* of RIF, we performed an average-linking hierarchical cluster analysis on those genes whose expression changed by more than 1,5-fold (either up or down). The object of the clustering was to group genes with similarly-regulated expression levels under *sub – MICs* of RIF (Fig. 1). We found that the genes that respond to *sub – MICs* of RIF fall into three general categories: those that are down-regulated in the presence *sub – MICs* of RIF (cluster **a,** 31 genes), those that show dose-dependent regulation (cluster **b**, 17 genes), and those that are up-regulated but not dependent on sub-inhibitory concentrations of RIF (cluster **c**, 38 genes). Cluster **b** contains the genes whose expression depends on *sub – MICs* of RIF. Some of

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the dose-regulated genes in this group are the flagellar gene, *flag* (HP0327); the pyrophosphokinase gene, *folk* (HP1036); the restriction enzyme gene, *hsdR* (HP0091); the response regulator gene, *ompR* (HP0166); the pathogenicity gene, *cag15* (HP0536); and the ribonuclease gene, *rnc* (HP1040).

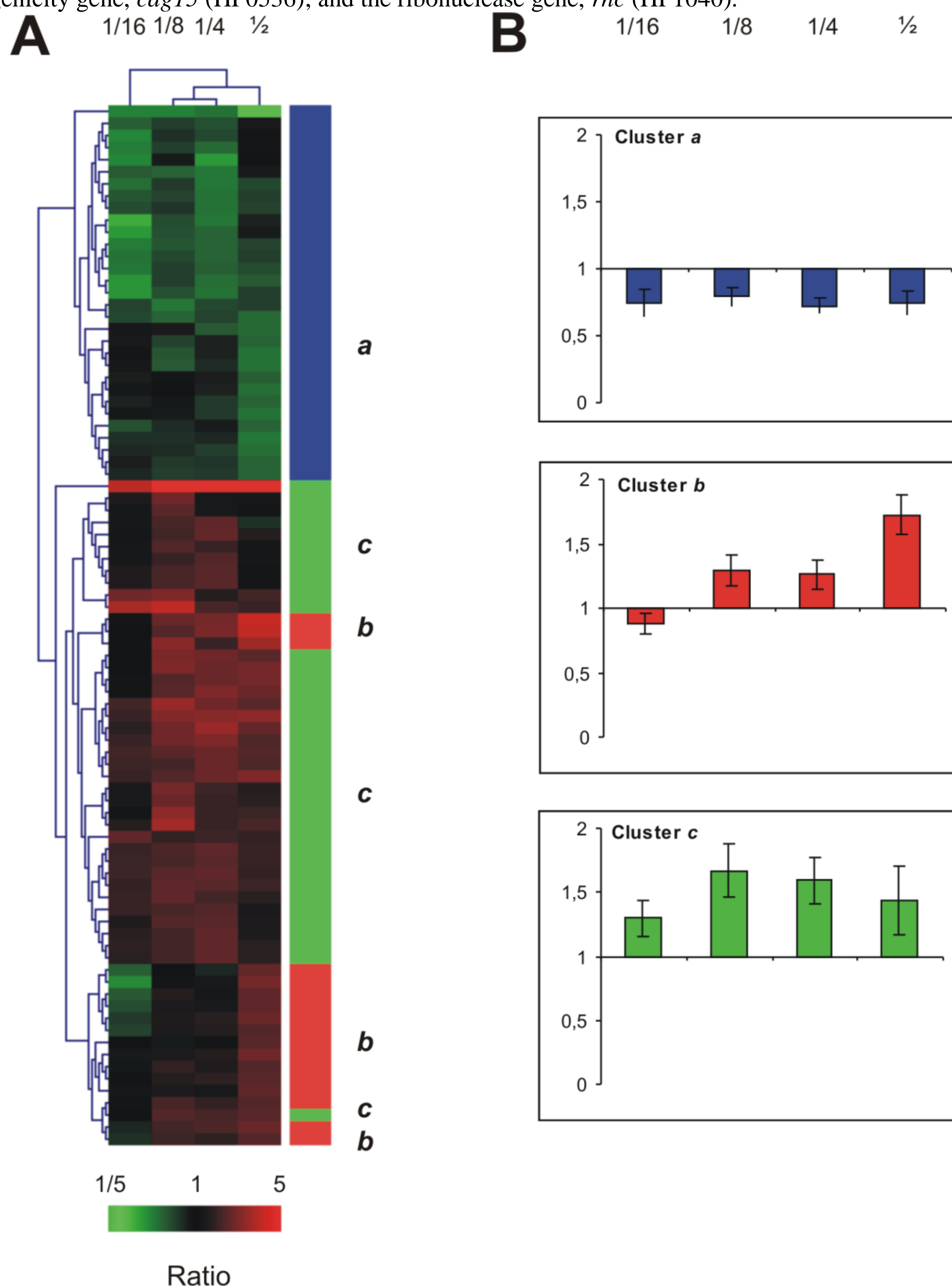

Figure 1. Cluster analysis of gene expression patterns at different *sub - MICs* of rifampicin (A). Hierarchical clustering was applied to the expression data from a set of 86 (of 1,576 total) genes measured under four *sub - MICs* of rifampicin: 1/16 MIC, 1/8 MIC, ¼ MIC, and ½ MIC. Transcript levels varied by at least 1.5-fold relative to the control condition. Data from 16 hybridizations were used. The color scale that was used to represent the expression ratios is shown on the bottom. The colored bars with labels *a* to *c* refer to the identified clusters of genes. Cluster **a** (31 genes): down-regulated genes in the presence *sub – MICs* of RIF*;* cluster **b:** dose-dependent regulated genes (17 genes); and cluster **c:** up-regulated, but not dependent on sub-inhibitory concentrations of RIF (38 genes). On the right is shown a profile bar graph for each cluster, in which the mean log ratio of that cluster in each experimental condition is plotted (B)

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Real-time RT-PCR analysis was used as an independent method to validate the microarray results, since it measures the accumulation of product during the linear phase of the PCR and is an accurate and reproducible approach to gene quantification [25, 26]. We quantified the transcripts of genes involved in biosynthesis/metabolism (*panD, pabB, ureI,* carbonic anhydrase*, atpC, acpP , pgsA, pyrC*), transcription/translation (*rpoB*), transport (*fecE* и *glnP*), protein fate (*groES*), and HP0759, HP1423, and HP1008. The real-time RT-PCR confirmed the up-regulation of *panD, pabB, ureI,* HP1186*, atpC, acpP, pgsA, pyrC*, *rpoB*, *fecE* и *glnP*, HP0759, HP1423, and HP1008 and the down-regulation of *groES* (Fig. 2).

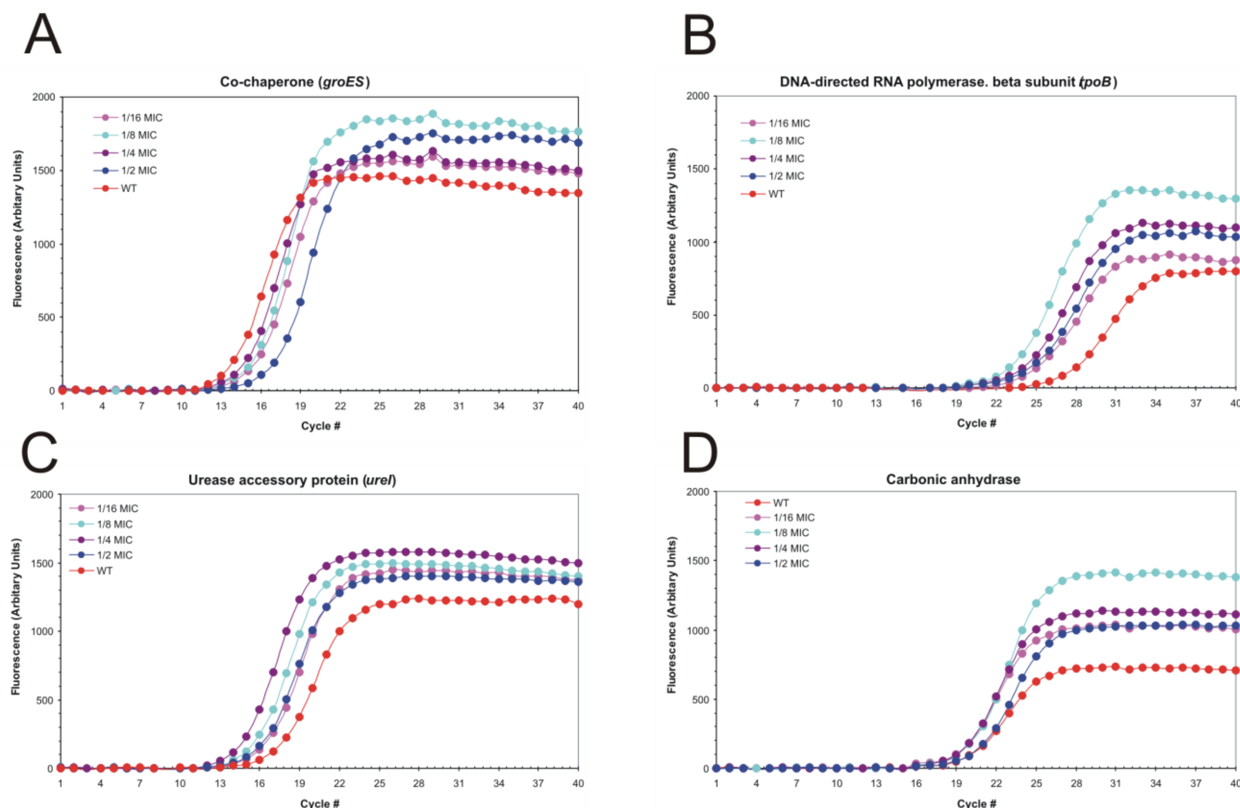

Figure 2. Real-time PCR. The cDNAs encoding the co-chaperone (*groES*) (A), beta subunit of the DNA-directed RNA polymerase (*rpoB*) (B), urease accessory protein (*ureI*) (C), and periplasmic carbonic anhydrase (D) from typical experiments of the microarray analysis at different subinhibitory concentration of rifampicin were subjected to PCR amplification. The lines represent amplifications from cDNAs generated from the RNAs isolated from *H. pylori* cultures incubated at 1/16 MIC (0,1 mg/L), 1/8 MIC (0,2mg/L), ¼ MIC (0,4 mg/L), ½ MIC (0,8 mg/L), and control conditions.

DISCUSSION

It was shown that rifampicin at subinhibitory concentrations alters the global bacterial transcription patterns [14, 28, 29]. Among the genes affected by antibiotics are those related to bacterial stress responses, as well as genes for accessory functions, such as motility and virulence (reviewed in 6 and 29). Previous work using cDNA-microarrays established a key role for *mfd* (*m*utation *f*requency *d*ecline, which encodes a transcription-repair coupling factor involved in DNA repair) in the resistance of *Campylobacter pylori* (closely related *Helicobacter*) to fluoroquinolines [15]. The mutation of *mfd* results in an approximately 100-fold reduction in the rate of spontaneous acquisition of ciprofloxacin resistance, while its overexpression elevates the mutation frequency. This result inspired us to use cDNA-microarrays to define the mechanism by which rifampicin at *sub-MICs* affects *H. pylori*.

Our analysis of *H. pylori* treated with *sub-MICs* of RIF allowed us to detect transcriptional variability in 86 of 1576 (5,4%) genes (Tables 2, 3). We found that the differentially regulated genes can be divided into three clusters: those whose expression is decreases at all *sub-MICs* of RIF (cluster **a**, 31 gene), dose-regulated genes that increase their expression with increasing concentration of antibiotic (cluster **b**, 17 genes), and those whose expression increases in response to antibiotic in a dose-independent fashion (cluster **c**, 38 genes) (Fig.1). Taking into account the S-shaped dependence of the mutation frequency on concentration, the most interesting genes are those in cluster **b**, whose expression is dose-dependent. In that cluster, we found genes with known functions: 7,8-dihydro-6-

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hydroxymethylpterin-pyrophosphokinase (*folK*), cag pathogenicity island protein (*cag15*), flagellar protein G (*flaG*), response regulator (*ompR*), ribonuclease III (*rnc*), and type II restriction enzyme R protein (*hsdR*).

Table 3. *H. pylori* genes of unknown function whose expression is induced or repressed in response at least to one subinhibitory concentrations of rifampicin

Expression	1/16 MIC	1/8 MIC	1/4 MIC	1/2 MIC	ORF ID
Induced	0.98	1.65	1.74	2.50	HP0016
	1.02	1.76	1.71	1.54	HP0057
	1.14	1.75	1.38	1.24	HP0058
	1.04	1.44	1.33	1.50	HP0081
	1.19	1.44	1.55	0.97	HP0085
	1.01	1.53	1.77	2.45	HP0129
	0.68	1.23	1.11	1.57	HP0167
	1.04	0.70	0.90	0.60	HP0218
	1.25	1.42	1.60	1.18	HP0236
	1.41	1.54	1.68	1.52	HP0287
	1.17	1.70	1.13	1.13	HP0365
	2.41	3.72	3.43	4.40	HP0412
	0.99	1.82	1.66	1.75	HP0560
	1.14	1.66	1.34	1.27	HP0673
	1.10	1.55	1.11	0.98	HP0674
	1.09	0.68	0.85	0.61	HP0730
	1.09	1.15	0.67	0.62	HP0784
	1.02	1.52	1.45	1.57	HP0833
	1.35	1.85	1.91	1.93	HP0882
	0.87	1.41	1.47	1.65	HP0990
	1.04	1.52	1.65	1.76	HP0999
	1.26	1.71	2.03	1.64	HP1003
	1.02	1.92	1.34	2.08	HP1051
	0.79	1.19	1.26	1.68	HP1154
	1.23	2.13	1.36	1.45	HP1163
	1.28	1.42	1.59	1.27	HP1187
	1.68	1.82	1.23	1.42	HP1211
	2.20	248	1.46	1.34	HP1219
	1.09	1.16	1.11	1.58	HP1265
	0.76	1.19	1.23	1.52	HP1276
	1.35	1.51	1.67	1.80	HP1333
	1.59	1.33	1.38	1.31	HP1396
Repressed					
	0.88	0.77	0.80	0.64	HP1542
	0.71	0.85	0.93	0.64	HP0001
	0.66	0.78	0.71	1.13	HP0187
	0.45	0.73	0.59	0.90	HP0206
	0.92	1.05	0.91	0.66	HP0221
	0.65	1.01	0.87	1.61	HP0342
	0.55	0.83	0.75	1.04	HP0427
	0.57	0.77	0.63	1.04	HP0430
	0.69	0.75	0.61	0.78	HP0445
	0.50	0.70	0.65	0.93	HP0622
	0.61	0.73	0.64	0.77	HP0667
	0.61	0.80	0.58	0.74	HP0732
	0.96	1.01	0.88	0.61	HP0762
	0.51	0.78	0.62	0.67	HP0817

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	0.88	0.96	0.79	0.65	HP0820
	0.59	0.78	0.66	0.73	HP0837
	0.67	0.65	0.59	0.93	HP0847
	0.55	0.92	0.50	1.02	HP0984
	0.72	0.81	0.60	0.75	HP1223
	0.51	0.71	0.60	0.78	HP1326

Of the genes whose expression is altered by *sub-MICs* of RIF, we found *ompR* (regulatory functions) and *rnc* (transcription) to be the most interesting. The *rnc* gene is interesting due to its role in RNA proccessing. Another gene, whose expression is modulated by *sub-MICs* of RIF, *ompR* (HP0166), is a part of the HP0165-HP0166 two-component system, which directly controls the transcription of genes that are involved in cellular pH homeostasis. Of the genes that are under the control of the response regulator HP0166, we found *ureI (HP0071), rnc (HP0662,) fecE (HP0888),* carbonic anhydrase *(HP1186),* and genes coding for proteins with unknown function (*HP1396, HP0667, HP0673, HP0674*) [27]. Carbonic anhydrase (HP1186) is an important periplasmic protein that catalyzes the conversion of CO_2, produced by urease, to HCO_3^- [19]. The protein encoded by the *ureI* gene, UreI, is a proton-gated urea entry channel that allows for the buffering of the periplasm of the organism with urease activity, which is essential for survival and growth in an acidic medium [22, 26] as well as for the colonization of mouse and gerbil stomachs [20, 23].

Another differentially expressed gene, *hypE* (HP0047), may also take part in cellular pH homeostasis, though it is not regulated by HP0166. The protein HypE (or HupE) is required for the expression of a catalytically active hydrogenase in many systems. The hydrogenase enzyme catalyses the redox equilibrium of $2H^+ + 2e^- \leftrightarrow H_2$. It is interesting that the expression of *HP0011* decreases with the addition of *sub - MICs*; this gene, codes for two proteins: heat shock protein HspA, which is needed for complete urease activity, and Hpn, which deposits Ni^{2+} metal from the center of active urease [11, 12]. The fact that the expression of genes involved in the maintenance of pH-homeostasis and regulated by HP0166 changes with *sub - MICs* perhaps indicates that the intracellular pH changes upon the addition of antibiotic.

The gene for the response regulator HP0166 has been shown to be essential for *H. pylori* cell growth, while the cognate histidine kinase HP0165 is not essential under *in vitro* culture conditions [3]. This result suggests that the HP0165-independent response regulator HP0166 is necessary for the transcription of target genes that are essential for *in vitro* growth, while an additional set of genes is activated or repressed under environmental conditions that trigger the histidine kinase activity of the sensor protein, HP0165. Hence, it is highly probable that the activation of HP0166 and functionally associated proteins in *H. pylori* indicates the switching on of other pH-independent mechanisms in response to the addition of *sub - MICs* of rifampicin. For instance, according to Beletskii (4), the frequency of induced mutations depends on level of transcription of the target cell and the physiologic state of cell. As shown above, a preincubation of *H. pylori* with *sub - MICs* of RIF causes an increase in RpoB expression; thus, it is no surprise that the frequency of mutations in all samples increases in comparison to the control. Obviously, the mutability of the microorganism depends on the functional activity of its mismatch repair system. In our study, we found that doses of rifampicin that are *sub - MIC* induce the synthesis of endonuclease III (HP0602), which protects the cell from the toxic and mutagenic action of alkylating agents.

Taking into account the fact that we failed to identify a direct correlation between *sub - MICs* of RIF and gene expression, it may be that the genes that are regulated by *sub - MICs* of RIF include not only stress-related genes that allow *H. pylori* to adapt to RIF but also genes that alter transcription through a direct interaction of rifampicin with the RNA polymerase β-subunit. It was shown in *S. typhimurium* and *E. coli* that subinhibitory concentrations of rifampicin can modulate (activate or repress) the transcription of a significant proportion of genes (5%) [14, 28], as detected by bacterial promoter-reporter constructs. It should be noted that the expression of *mfd* (HP1541), which plays a key role in the development of fluoroquinolone resistance in *C. jejuni,* does not change in *H. pylori* after exposure to *sub - MICs* of RIF. Thus, the mechanism by which the frequency of mutations increases during a selection with *sub - MICs* of RIF in *H. pylori* remains unclear. Considering that, in clinical isolates *of H. pylori*, hypermutable strains are often found [5], we suppose that, along with the modulation of gene expression, a selection of cells with a hypermutable phenotype simultaneously takes place.

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РЕЗЮМЕ

Субингибирующие концентрации (суб-МИК) антибиотиков не оказывают бактерицидного действия на бактерии, но они могут оказывать влияние на важные аспекты бактериальной клеточной функции, такие как адгезия к клеткам-хозяевам, восприимчивость к защитным механизмам хозяина, ингибировать ферменты и влиять на выработку токсинов и т.п. Для того, чтобы понять, как *H. pylori* справляется с внешним стрессом и что способствует появлению рифампицин-устойчивых мутантов РИФ, мы использовали ДНК-микрочипы для сравнения профилей экспрессии генов *H. pylori* в присутствии и отсутствии субингибирующей концентрации рифампицина (1/16 МИК (0,1 мг/Л), 1/8 МИК (0,2 мг/Л), ¼ МИК (0,4 мг/Л) и ½ MIC (0,8 мг/Л)). Было найдено, что экспрессия 57 генов (из 1576 проанализированных генов) увеличилась более чем ≥1,5 раза, а экспрессия 29 генов была достоверно снижена более чем ≤ 1,5 раза (р <0,05), когда *H. pylori* инкубировали в присутствии субингибирующих концентраций рифампицина (РИФ). Однако, не было обнаружено никакой дозозависимой корреляции между суб-МИК РИФ и экспрессией генов. Мы пришли к выводу, что изменения транскрипционных профилей *H. pylori* после воздействия суб-МИК РИФ происходит в основном за счет прямого взаимодействия рифампицина и β-субъединицы РНК-полимеразы. Полагаем, что субингибирующие концентрации рифампицина могут быть причиной увеличения количества гипермутабельных клеток в *H. pylori* популяции.

Ключевые слова: *Helicobacter pylori*, отбор, микроэррей, *rpoB*.

ТҮЙІН

Антибиотиктердің субингибициялаушы шоғырланулары (суб-МИК) бактерияларға бактерицидті әрекет жасамайды, бірақ олар иелік етуші жасушаларға жабысып қалу, иенің қорғаныш тетіктеріне қабылдағыштық, ферменттерді тежеу және уыт өндіруге әсер ету және тағы сол сияқты бактериялық жасушалық функцияның маңызды аспектілеріне әсерін тигізуі мүмкін.*H. Pylori* сыртқы күйзелісті қалай жеңетінін және рифампицинге тұрақты РИФ мутанттарының пайда болуына мүмкіндік туғызатынын түсіну үшін, біз рифампицинсубингибициялаушы шоғырлануының қатысуымен және оның жоғында *H. Pylori* тектері дәлдігінің профильдерін салыстыру үшін ДНК-микрочиптерді пайдаландық (1/16 МИК (0,1 мг/Л), 1/8 МИК (0,2 мг/Л), ¼ МИК (0,4 мг/Л) и ½ MIC (0,8 мг/Л)). *H. Pylori*-ді рифампициннiңсубингибициялаушы шоғырлануларының қатысуымен инкубациялағанда (РИФ)57 тектің дәлдігі (1576 анализден өткен тектерден) ≥1,5 еседен астам артқандығы, ал 29 тектің дәлдігі ≤1,5 еседен астам (р <0,05) анық кемігендігітабылды. Алайда суб-МИК РИФ және тектер дәлдігі арасында ешқандай дозалық тәуелді корреляция байқалған жоқ. Біз суб-МИК РИФ әсерінен кейін *H. Pylori*-дің транскрипциялық профильдерінің өзгеруі негізінен рифампициннің және β-суббірлігі РНК-полимеразасының тікелей өзара іс-әрекеті есебінен жүреді деген қорытындыға келдік. Біз рифампициннiңсубингибициялаушы шоғырланулары. *H. Pylori* популяциясында гипермутабельді жасушалар санының көбею себебі болуы мүмкін деп ойлаймыз.

Кілтті сөздер: *Helicobacter pylori*, іріктеу, микроэррей, *rpoB*.