INFECTION AND IMMUNITY, June 2003, p. 3529–3539 Vol. 71, No. 6
0019-9567/03/$08.00+0 DOI: 10.1128/IAI.71.6.3529–3539.2003

pH-Regulated Gene Expression of the Gastric Pathogen *Helicobacter pylori*

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Received 31 October 2002/Returned for modification 28 January 2003/Accepted 10 March 2003

Colonization by the gastric pathogen *Helicobacter pylori* has been shown to be intricately linked to the development of gastritis, ulcers, and gastric malignancy. Little is known about mechanisms employed by the bacterium that help it adapt to the hostile environment of the human stomach. In an effort to extend our knowledge of these mechanisms, we utilized spotted-DNA microarrays to characterize the response of *H. pylori* to low pH. Expression of approximately 7% of the bacterial genome was reproducibly altered by shift to low pH. Analysis of the differentially expressed genes led to the discovery that acid exposure leads to profound changes in motility of *H. pylori*, as a larger percentage of acid-exposed bacterial cells displayed motility and moved at significantly higher speeds. In contrast to previous publications, we found that expression of the bacterial virulence gene *cagA* was strongly repressed by acid exposure. Furthermore, this transcriptional repression was reflected at the level of protein accumulation in the *H. pylori* cell.

Since its isolation from gastric biopsy samples in 1982 by Marshall and Warren (44), the small gram-negative bacterium *Helicobacter pylori* has been shown to infect over 50% of the world's population (45). While transmission of the bacterium between humans or from an unknown environmental reservoir is still poorly characterized, it is now understood that colonization generally results in a persistent infection that, unless specifically treated, lasts the lifetime of the infected individual (12). This has profound clinical importance, as *H. pylori* colonization has been shown to be associated with multiple forms of gastric disease. These range in severity from mild gastritis to duodenal and gastric ulcers and two forms of gastric malignancy, mucosa-associated lymphoid tissue lymphoma and adenocarcinoma (13, 24, 59).

In order to establish a successful infection, bacteria that colonize within the dynamic substrate of a human host must have the ability to adapt to and modify gene expression in response to changes in the host environment. A thorough understanding of the survival mechanisms employed by *H. pylori* during colonization of the stomach remains lacking, but one of the most obvious challenges is the constant fluctuations in pH that occur. The gastric lumen can reach a pH of 2.0 during the course of digestion, and while the mucus layer overlaying the gastric epithelium, the site of colonization of *H. pylori*, generally remains more neutral, the bacteria no doubt face fluctuations in pH that must be sensed and responded to (75). Because of this, mechanisms involved in acid resistance (AR) have been the focus of considerable study. AR has been shown to be intricately linked with *H. pylori*'s ability to produce copious amounts of urease (55). The urease enzyme catalyzes the hydrolysis of urea to carbon dioxide and ammonia and helps maintain a proton motive force across the inner membrane of the bacterium (54, 64). It also leads to the extrusion of the basic ammonia molecules into the surrounding environment, thus buffering the bacterial microenvironment. Urease enzymatic activity is crucial for colonization and persistence in the stomach (6, 25, 28, 79).

In addition to urease, genes for other factors have also been shown to be important for AR. These include *wbcJ*, which is involved in the assembly of mature lipopolysaccharide (49); *pldA*, which undergoes phase variation leading to changes in the lipid composition of the outer membrane that affect AR (74); *recA*, which is involved in DNA damage repair (77); *rocF*, which is required for the arginase activity that hydrolyzes L-arginine to L-ornithine and urea (47); *atpD* and *atpF*, which encode subunits of the F_1F_0-ATPase (9, 48); and *fur*, which encodes the ferric iron uptake regulator and was recently identified as the first known transcriptional regulator required for growth in acidic conditions (10). The activities of these gene products exemplify the fact that a diverse range of factors, active at many subcompartments in the cell, are involved in the adaptive response to acid.

Microarray technology is a powerful tool for analyzing the transcriptional response of microbes to changes in environmental conditions, such as those that mimic in vivo conditions that pathogens would encounter within the context of their host. As such, two previous microarray-based studies have determined some of the transcriptional changes in *H. pylori* as it adapts to acid stress (3, 7). In both studies, only a single time point after exposure to acid conditions was examined. There was no overlap between the genes identified in the two studies, suggesting that neither screen was comprehensive or, alternatively, that the two sets of acid conditions examined result in changes in nonoverlapping sets of genes.

To better understand adaptive mechanisms utilized by *H. pylori* within the context of the host environment, we utilized spotted-DNA microarrays to characterize in a temporal man-

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ner the global changes in gene expression in response to low pH in the pathogenic *H. pylori* strain G27. Direct comparison to transcriptional levels of the starting culture revealed that expression of many genes was rapidly altered and remained altered for the duration of the acid exposure. Approximately 7% of the predicted open reading frames (ORFs) were significantly up or down regulated. Among these were many factors whose expression had previously been shown to be affected by low pH, as well as many factors whose pH-regulated gene expression had not previously been identified. Differential regulation of a number of the components of the flagellar regulon led us to analyze the effect of acid exposure on motility of *H. pylori*, and a profound increase in both the percentage and speed of motile bacteria after acid exposure was noted. In contrast to previous reports (3, 37), expression of the virulence gene *cagA* was repressed by acid exposure and, accordingly, a reduced level of CagA was found in the bacterial cell. Finally, mutation of the acid-repressed *ansB* gene, while showing no obvious effect on in vitro AR, resulted in a strain that was significantly less able to stably infect within the context of the Mongolian gerbil model of *H. pylori* gastric colonization.

MATERIALS AND METHODS

Bacterial strains and growth. The *H. pylori* strains G27 (18) and B128 (60) were maintained as frozen stocks at −80°C in brain heart infusion media supplemented with 20% glycerol and 10% fetal bovine serum (FBS). Bacteria were grown on horse blood agar (HBA) plates containing 4% Columbia agar base (Oxoid), 5% defibrinated horse blood (HemoStat Labs, Dixon, Calif.), 0.2% β-cyclodextrin (Sigma), 10 μg of vancomycin (Sigma)/ml, 5 μg of cefsulodin (Sigma)/ml, 2.5 U of polymyxin B (Sigma)/ml, 50 μg of cycloheximide (Sigma)/ml, 5 μg of trimethoprim (Sigma)/ml, and 8 μg of amphotericin B (Sigma)/ml under microaerophilic conditions at 37°C. A microaerobic atmosphere was generated by using a CampyGen sachet (Oxoid) in a gas pack jar. For liquid culture, *H. pylori* was grown in brucella broth (Difco) containing 10% FBS (Gibco/BRL) with shaking in a microaerobic environment.

Acid exposure and RNA isolation. An overnight liquid culture of G27 (optical density at 600 nm [OD_{600}] = 0.5 to 1.0) was harvested by centrifugation and resuspended in pH 5.0 brucella broth supplemented with 10% FBS. The pH of the media was adjusted by using concentrated hydrochloric acid prior to resuspension. Bacterial samples collected prior to acid exposure represent the zero time point, and samples were harvested after 30, 60, 90, and 120 min of acid exposure. At each time point, aliquots were removed from the pH 5.0 media and were plated to determine the numbers of CFU and were harvested on a 0.45-μm-pore-size cellulose filter by vacuum filtration. Filters containing the *H. pylori* cells were immediately frozen in liquid nitrogen and were subsequently used for isolation of bacterial RNA.

H. pylori RNA was isolated by using TRIzol reagent (Gibco/BRL) as previously described (52). RNA concentration was quantitated by determination of the absorbance at 260 and 280 nm, and RNA integrity was verified by visualization on a 1% agarose gel.

Microarray hybridization and analysis. Equal concentration of each test RNA (T = 30, 60, 90, and 120 min from biologically independent experiments) and the reference RNA (T = 0) were used for cDNA synthesis in a standard reverse transcriptase reaction by using Superscript II (Invitrogen) and Panorama *H. pylori* cDNA labeling primers (SigmaGenosys). Synthesized cDNA was purified by using Qia-Quick PCR purification columns (Qiagen) according to the manufacturer's instructions and subsequently were indirectly labeled with Cy5 and Cy3 fluorophores as previously described (62). Individual Cy5 and Cy3 reactions were combined, and unincorporated dye was removed by using a Qia-Quick PCR column according to the manufacturer's instructions. The eluate from the columns was concentrated by evaporation in a Speed Vac and was resuspended in 11 μl of Tris-EDTA. One microliter of 25-mg/ml yeast tRNA, 2.55 μl of 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 0.45 μl of 10% sodium dodecyl sulfate were added to each of the labeled probes. These were heated to 99°C for 2 min, cooled briefly, and then added to the *H. pylori* microarray for hybridization and stringency washes as has been previously described (62). The hybridized slides were scanned and analyzed by using an Axon 4000A scanner and the GENEPIX 3.0 software (Axon).

Data were collated with the Stanford University Microarray Database (67). Spots showing obvious abnormalities were excluded from analysis. Spot quality was further filtered by requiring that the Cy3 mean spot intensity-to-Cy5 median background intensity ratio be greater than or equal to 2.5 and the Cy5 mean spot intensity-to-Cy3 median background intensity ratio be greater than or equal to 2.5. Genes were filtered by providing that the log (base 2) of the red-to-green normalized ratio be greater than 1.8 standard deviations from the mean ratio in at least one array for each experimental set. Biologically independent experiments were analyzed separately, and the data were collapsed to reveal factors whose expression was consistently altered by acid exposure. All microarray data generated by this study are publicly available at http://genome-www.stanford.edu/microarray/.

Motility analysis. A liquid culture of G27 was harvested by centrifugation and split into two portions: one was resuspended in pH 7.0 brucella broth plus 10% FBS and the other in pH 5.0 brucella broth plus 10% FBS. Motility was monitored by live phase-contrast microscopy. A Hamamatsu C2400 video charge-coupled device camera was used to record via an Argus-20 image processor onto S-VHS video. Resulting images were digitized into NIH Object Image for the generation of time-lapse films. Adobe Photoshop was used to assemble the figures. To trace the speed of movement of the bacteria, the TRACE function of the Argus-20 image processor was used and the individual length of the traces was calculated by using NIH Object Image.

Δ*ansB* construction and characterization. A deletion-insertion of the *ansB* coding sequence (HP0723) was constructed by allelic exchange in strain B128. The allelic exchange vector was constructed by amplification of an *ansB*-upstream fragment by using primers Hp0723-1 (GCGGCCGCAATCCAATCAAGCAGAAA) and Hp0723-2 (TCTAGAGAAGAGTATTGAAAGATT) and of an *ansB*-downstream fragment by using primers Hp0723-3 (TCTAGACATAACCTGCCCCTTCAA) and Hp0723-4 (GGTACCAACGATCGCTACAACCCCA). The primers incorporate *Xba*I and *Not*I sites and *Xba*I and *Kpn*I sites, respectively (underlined), that were used for subsequent directional cloning. PCR products were subcloned into the pCR2.1-TOPO vector (Invitrogen), and fragments were then removed by restriction enzyme digestion (enzymes mentioned above) and were directionally ligated with a kanamycin resistance cassette into a pSK-derived suicide vector (36). The resulting construct was verified by restriction enzyme digestion with appropriate enzymes and was subsequently used for natural transformation of *H. pylori*. Allelic exchange mutants were selected by plating on HBA plates supplemented with 25 μg of kanamycin/ml, and the proper deletion-insertion was confirmed by Southern blotting.

In vitro growth curves were conducted by inoculation of brucella broth supplemented with 10% FBS to a starting OD_{600} of approximately 0.06 to 0.1 with an overnight-grown culture of the wild type or *ansB* mutant followed by subsequent determination of the OD_{600} at the time points indicated in Fig. 3. In vitro competition assays were conducted by mixing equal numbers of CFU of the *ansB* and wild-type strain in brucella broth plus 10% FBS, which was followed by growth with shaking in a microaerobic environment. Relative numbers of CFU of each strain were then determined by plating on HBA alone and HBA supplemented with kanamycin.

Immunoblot detection of CagA. The relative concentration of the CagA protein was determined by collection of bacterial cells that had undergone an acid exposure as described for the microarray analysis above. Bacterial cells were lysed by boiling in standard 1× sodium dodecyl sulfate buffer, and the protein concentration was determined using a BCA kit (Pierce). An equal amount of protein from each sample was separated on a 6% polyacrylamide gel by electrophoresis, and the proteins were transferred by using a semidry transfer apparatus (E&K Scientific) to nitrocellulose membrane. The resulting blot was probed with a primary mouse monoclonal anti-CagA antibody (Austra Biological) and a secondary anti-mouse immunoglobulin G antibody conjugated to Alexa 660 (Molecular Probes). Resulting bands were visualized by scanning with an Odyssey scanner, and relative protein concentrations were determined by scanning densitometry with the same program (Licor).

Gerbil infections. Four- to 8-week-old male Mongolian gerbils (Harlan, Indianapolis, Ind.) were fasted for 20 h prior to infection and were subsequently infected with 0.5 ml of a 1:1 mix of liquid grown wild-type B128 and Δ*ansB* bacteria via oral gavage. This represents an inoculum of approximately 10^8 to 10^9 total bacteria. Infections were allowed to proceed for 2.5 to 3 weeks, and then animals were sacrificed, the glandular portion of the stomach was excised and homogenized with a mechanical homogenizer, and the numbers of CFU were determined by plating on HBA alone and HBA supplemented with kanamycin. The relative number of wild-type bacteria was determined by subtraction of the number of kanamycin-resistant colonies from the total number of colonies grow-

ing on the plain-HBA plates. The competitive index was determined by division of the number of mutant bacteria by the number of wild-type bacteria followed by corrections for deviations from an input ratio of 1:1.

RESULTS AND DISCUSSION

Microarray analysis of pH-regulated gene expression of G27. To reveal dynamic changes that occur in response to low pH, it was necessary to ensure that *H. pylori* strain G27 would remain viable throughout the course of the experiment. This was a critical experimental parameter, as decreased viability at later time points could greatly affect the transcriptional profiles. Consequently, survival curves for G27 were determined in pH 5.0 brucella broth supplemented with 10% FBS and revealed virtually full survival of the strain throughout the 2-h exposure (data not shown). The pH of the media also remained unchanged, suggesting that these conditions would be useful for determination of expression profiles of the strain.

An overnight culture of G27 was harvested by centrifugation and resuspended in pH 5.0 brucella broth media as described in Materials and Methods. Samples were taken prior to resuspension and at T = 30, 60, 90, and 120 min thereafter. Two independent experiments were conducted on separate days, and total RNA was prepared from all 10 samples and utilized for transcriptome analysis. For each experiment, cDNA synthesized from RNA from the zero time point was utilized as the reference and was labeled with Cy3 while cDNA synthesized from RNA harvested after exposure to acid was labeled with Cy5. Each Cy5 and Cy3 pair was hybridized to a spotted-DNA microarray representing 98.9% of the unique ORFs found in both of the sequenced *H. pylori* strains (5, 78). Each DNA fragment is additionally spotted in duplicate on the array, thus allowing for technical replicates within a single hybridization (62). Ratios of each Cy5 and Cy3 fluorophore for each spot were obtained by scanning with an Axon scanner and represent the changes in total mRNA content after exposure to the acid stimulus. Data for each experiment were filtered as described in Materials and Methods, and genes showing altered patterns of expression in both experiments are schematically depicted in Fig. 1 and are listed in Table 1 and in the supplementary information at http://falkow.stanford.edu/whatwedo/supplementarydata/.

Classification of acid-regulated genes. The majority of genes that displayed altered expression upon shift to acidic pH showed a net increase in transcription; 71 of the 118 showed at least twofold up regulation at one of the time points. Sorting of the acid-regulated genes into the functional classes defined by The Institute for Genomic Research reveals that the largest class that shows altered expression is composed of hypothetical ORFs of unknown function; 36 of the 118 fall into this group. This represents approximately one-third of the identified genes and highlights our minimal understanding of many of the gene products encoded by *H. pylori*. Approximately 45% of the assigned ORFs of *H. pylori* are predicted to encode genes that are hypothetical or conserved hypothetical or of unknown function (78). This is not unique to *H. pylori*, however, as >50% of all of the predicted ORFs in the 87 completed genomic sequences are predicted to encode hypothetical or conserved hypothetical genes or genes of unknown function (Comprehensive Microbial Resource database at http://www.tigr.org/tigr-scripts/CMR2/CMRHomePage.spl). A goal of future research will be to determine the functional role of these many factors in the life cycle of *H. pylori* and, in particular, the roles of the genes in Table 1 in its adaptation to acid stress.

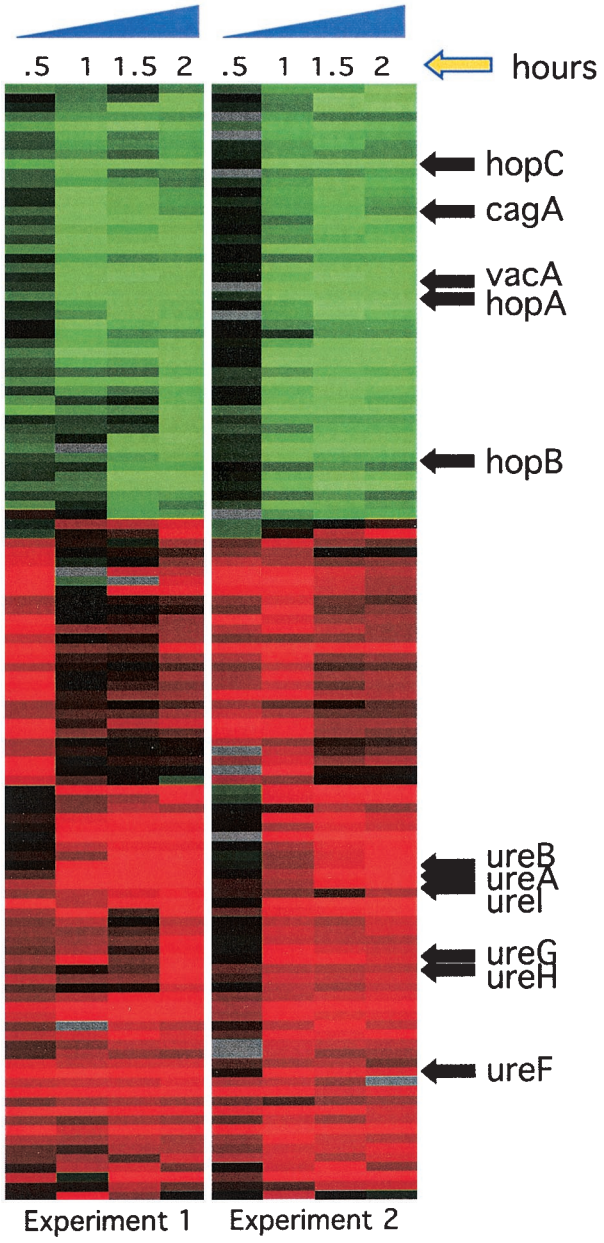

FIG. 1. Microarray analysis of the low-pH response of *H. pylori*. Cluster diagram showing the expression profile of the 118 genes meeting the filter criteria as explained in Materials and Methods after shift to pH 5.0. Two independent experiments are depicted, and relative expression patterns are shown for each time point. Red indicates an increase in expression, while green indicates reduced expression. Representative genes are listed and their relative location indicated by an arrow. A complete list of the regulated factors and the relative changes in expression can be found in Table 1 and as supplementary information at http://falkow.stanford.edu/whatwedo/supplementarydata/.

Other functional classes with the largest numbers of factors altered by exposure to acid pH include those predicted to have a role in assembly and/or maintenance of the cell envelope and

TABLE 1. pH-regulated genes of *H. pylori*[a]

Expression	Class	Gene designation		Gene and function	Change (*n*-fold) at:			
		TIGR	ASTRA		$T = 30$	$T = 60$	$T = 90$	$T = 120$[b]
Induced	Amino acid biosynthesis	HP1282	JHP1203	Anthranilate synthase component, *trpE*	2.4	2.5	2.5	2.7
		HP0106	JHP0098	Cystathionine gamma-synthase, *metB*	2.3	1.8	1.8	3.5
		HP1050	JHP0375	Homoserine kinase, *thrB*	1.9	2.3	3.0	3.1
		HP0290	JHP0275	Diaminopimelate decarboxylase (dap decarboxylase), *lysA*	1.2	2.4	2.9	2.9
		HP0380	JHP1001	Glutamate dehydrogenase, *gdhA*	2.4	1.2	1.3	2.0
	Biosynthesis of cofactors, prosthetic groups, and carriers	HP0306	JHP0291	Glutamate-1-semialdehyde 2,1-aminomutase, *hemL*	1.9	1.9	1.6	2.6
		HP0755	JHP0692	Molybdopterin biosynthesis protein, *moeB*	1.7	1.6	2.2	2.2
	Cell envelope and surface structures	HP1155	JHP1082	UDP-*n*-acetylglucosamine-*n*-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol *n*-acetylglucosamine transferase, *murG*	3.4	1.4	1.3	1.9
		HP0743	JHP0680	Rod shape-determining protein, *mreB*	1.6	1.9	2.4	2.5
		HP0295	JHP0280	Flagellin B homolog (HAP3)	4.2	1.4	1.9	2.0
		HP1119	JHP1047	Flagellar hook-associated protein 1 (HAP1), *flgK*	3.9	1.7	1.3	2.1
		HP1559	JHP1467	Flagellar basal-body rod protein (proximal rod protein), *flgB*	3.8	ND[c]	2.5	2.4
		HP0115	JHP0107	Flagellin, *flaB*	3.5	1.0	1.4	1.4
		HP0870	JHP0804	Flagellar hook, *flgE*	3.0	1.1	1.5	1.9
		HP1558	JHP1466	Flagellar basal-body rod protein (proximal rod protein), *flgC*	2.0	1.1	1.3	1.6
		HP1031	JHP0393	Flagellar motor switch protein, *fliM*	1.8	2.3	1.1	2.3
		HP1122	JHP1051	Anti-σ28 factor, *flgM*	2.7	1.1	1.3	1.6
	Cellular processes	HP1332	JHP1252	Cochaperone and heat shock protein, cochaperone with DnaK, *dnaJ*	4.0	4.0	3.0	3.1
	Central intermediary metabolism	HP0069	JHP0064	Urease accessory protein, *ureF*	3.0	4.0	3.4	3.5
		HP0071	JHP0066	Urease accessory protein, *ureI*	2.2	4.1	6.4	5.5
		HP0068	JHP0063	Urease accessory protein, *ureG*	1.8	2.1	1.8	3.0
		HP0067	JHP0062	Urease accessory protein, *ureH*	1.7	2.2	1.5	3.0
		HP0073	JHP0068	Urease alpha subunit, *ureA*	1.7	3.8	7.2	6.9
		HP0072	JHP0067	Urease beta subunit, *ureB*	1.2	3.4	7.1	7.2
	DNA metabolism, restriction and modification	HP1121	JHP1050	Cytosine-specific DNA methyltransferase, BSP6IM	2.2	1.3	1.2	1.6
	Energy metabolism	HP0294	JHP0279	Aliphatic amidase energy metabolism, *amiF*	4.1	2.4	11	9.5
		HP1238	JHP1159	Aliphatic amidase energy metabolism, *amiE*	2.4	0.8	9.0	7.5
			JHP585	Putative 3-hydroxyacid dehydrogenase	1.0	3.2	4.2	10.1
		HP0642	JHP0586	NAD(P)H-flavin oxidoreductase	1.1	5.7	8.1	8.1
	Fatty acid and phospholipid metabolism and biosynthesis	HP0557	JHP0504	Acetyl-coenzyme A carboxylase, *accA*	1.4	1.4	2.3	1.7
	Hypothetical gene	HP1233	JHP1154		5.7	0.6	ND	2.8
		HP1154	JHP1081		5.1	1.1	1.4	1.9
		HP0367	JHP1014		4.6	1.5	2.3	2.6
		HP0119			4.5	0.9	4.1	3.7
		HP0118	JHP0110		4.3	1.7	1.7	3.2
		HP1327	JHP1247		3.9	1.2	1.1	1.0
		HP1187	JHP1113		3.8	2.6	3.7	3.6
		HP1076	JHP0349		3.6	1.8	1.5	1.6
		HP0219	JHP0205		2.9	3.1	3.4	3.5
		HP1173	JHP1100		2.8	4.0	5.9	4.9
		HP1188			2.8	0.8	1.0	1.9
		HP1022	JHP0402		2.2	2.1	2.4	2.1
		HP1440	JHP1333		2.1	1.1	1.2	1.3
		HP1457	JHP1350		2.0	1.4	1.2	1.6
		HP0018	JHP0016		1.7	2.1	1.7	2.6
		HP0554	JHP0501		1.7	2.2	2.0	1.9
		HP0719	JHP0657		0.8	2.0	2.3	2.8
		HP0641	JHP0584		1.4	1.9	5.4	5.7
		HP1331	JHP1251	Conserved hypothetical integral membrane protein	2.7	2.5	2.6	2.6
		HP0228	JHP0213	Conserved hypothetical integral membrane protein	2.1	3.8	1.5	4.1
		HP1225	JHP1146	Conserved hypothetical integral membrane protein	2.1	1.6	1.7	2.0
		HP0318	JHP0301	Conserved hypothetical protein	2.0	3.2	1.8	3.8
		HP1430	JHP1323	Conserved hypothetical ATP-binding protein	1.8	ND	2.5	2.2
		HP1507	JHP1400	Conserved hypothetical ATP-binding protein	1.7	1.4	1.7	4.0
		HP0759	JHP0696	Conserved hypothetical integral membrane protein	1.5	2.3	2.7	1.8
		HP1020	JHP0404	Conserved hypothetical protein	1.3	3.0	3.1	2.9
	Purines, pyrimidines, nucleosides and nucleotides	HP0757	JHP0694	β-alanine synthetase homolog	2.3	2.6	3.0	2.7
	Regulatory functions	HP1021	JHP0403	Putative transcriptional regulator	1.4	2.9	3.6	3.9
		HP0278	JHP0263	Guanosine-5′-triphosphate-3′-diphosphate pyrophosphatase, *gppA*	0.7	1.3	1.5	3.4
	Transcription, transcription factors and translation	HP0550	JHP0497	Transcription termination factor Rho, *rho*	2.6	1.6	1.0	0.7
		HP1203	JHP1126	Transcription termination factor NusG, *nusG*	1.8	2.0	2.2	2.0
		HP0123	JHP0113	Threonyl-tRNA synthetase, *thrS*	2.0	1.8	2.1	2.0
		HP1019	JHP0405	Serine protease, *htrA*	2.5	3.4	4.2	3.9
	Transport and binding proteins	HP1171	JHP1098	Glutamine ABC transporter, ATP-binding protein, *glnQ*	1.1	1.9	2.3	2.1
		HP1172	JHP1099	Glutamine ABC transporter, periplasmic glutamine-binding protein, *glnH*	1.0	1.8	2.0	3.6

Continued on following page

TABLE 1—*Continued*

Expression	Class	Gene designation		Gene and function	Change (*n*-fold) at:			
		TIGR	ASTRA		$T = 30$	$T = 60$	$T = 90$	$T = 120$[b]
		HP0471	JHP0423	Glutathione-regulated potassium efflux system, *kefB*	2.1	1.4	1.2	1.2
		HP1339	JHP1258	Biopolymer transport protein, *exbB*	3.0	1.9	1.8	2.0
		HP1340	JHP1259	Biopolymer transport protein, *exbD*	2.3	1.4	0.9	2.1
		HP0876	JHP0810	Iron-regulated OMP, *frpB*	1.4	3.3	1.9	1.2
		HP0715	JHP0653	ABC transporter, ATP-binding protein	2.2	1.0	1.2	1.4
		HP1432	JHP1321	Putative histidine and glutamine-rich metal-binding protein	2.0	2.4	2.8	3.1
		HP1465	JHP1358	ABC transporter, ATP-binding protein, HI1087	1.7	1.3	1.3	2.6
Repressed	Amino acid biosynthesis	HP1038	JHP0386	3-Dehydroquinase type II, *aroD*	0.6	0.4	0.6	0.5
		HP0695	JHP0633	Hydantoin utilization protein A, *hyuA*	1.0	0.3	0.4	0.4
	Cell envelope	HP0722	JHP0659	Putative OMP, *hopO*	0.7	0.1	0.1	0.1
		HP0638	JHP0581	OMP, *omp13* (*hopH*)	0.7	0.7	0.4	0.4
		HP0913	JHP0849	OMP, *hopB*	0.7	0.5	0.4	0.4
		HP0317		OMP, *omp9* (*hopU*)	0.7	0.4	0.5	0.4
		HP0725	JHP0662	Putative OMP, *hopP*	0.7	0.7	0.7	0.4
		HP1177	JHP1103	OMP, *omp27* (*hopQ*)	0.6	0.1	0.1	0.1
		HP0009	JHP0007	OMP, *hopZ*	0.6	0.8	0.4	0.3
		HP1469	JHP1362	OMP, *omp31* (*horJ*)	0.6	0.5	0.3	0.3
		HP0229	JHP0214	OMP, *hopA*	0.5	0.2	0.2	0.2
		HP1501	JHP1394	OMP, *omp32* (*horK*)	0.5	0.1	0.1	0.1
		HP0912	JHP0848	OMP, *hopC*	0.4	0.3	0.3	0.2
		HP1167	JHP1094	Conserved putative OMP, *hofH*	0.5	ND	0.2	0.3
	Cellular processes	HP0010	JHP0008	Chaperone and heat shock protein, 60-kDa chaperone, *groEL*	0.7	0.4	0.4	0.2
		HP0109	JHP0101	Chaperone and heat shock protein, 70, 70-kDa chaperone, *dnaK*	0.6	0.4	0.4	0.2
		HP0110	JHP0102	Cochaperone and heat shock protein, 24-kDa chaperone, *grpE*	0.5	0.4	0.4	0.2
		HP0887	JHP0819	Vacuolating cytotoxin, *vacA*	0.8	0.3	0.3	0.2
		HP0547	JHP0495	Cag pathogenicity island protein, *cag26* (*cagA*)	0.8	0.3	0.4	0.4
	Central intermediary metabolism	HP1186	JHP1112	Carbonic anhydrase	1.2	0.5	0.7	0.3
	DNA metabolism, DNA replication, recombination, and repair	HP1460	JHP1353	DNA polymerase III alpha subunit, *dnaE*	0.6	0.3	0.4	0.5
	Energy metabolism	HP0056	JHP0048	Δ-1-pyrroline-5-carboxylate dehydrogenase, *putA*	0.8	0.4	0.5	0.5
		HP0723	JHP0661	L-asparaginase II energy metabolism, *ansB*	0.5	0.8	0.4	0.5
		HP1458	JHP1351	Thioredoxin	0.8	0.4	0.3	0.4
		HP1101	JHP1027	Glucose-6-phosphate dehydrogenase, *g6pD*	0.5	0.6	0.8	0.5
	Fatty acid and phospholipid metabolism	HP0871	JHP0805	CDP-diglyceride hydrolase, *cdh*	0.6	0.4	0.5	0.4
		HP1045		Acetyl-coenzyme synthetase, *acoE*	0.7	0.3	0.4	0.4
	Hypothetical gene	HP1002			1.4	0.9	0.4	0.5
		HP0637	JHP0580		0.7	0.7	0.4	0.4
		HP0097	JHP0089		0.6	0.4	0.4	0.3
		HP1527	JHP1416		0.6	0.5	0.6	0.4
		HP1322	JHP1242		0.6	0.5	0.5	0.5
		HP0111	JHP0103		0.4	0.5	0.5	0.2
		HP1175	JHP1102	Conserved hypothetical integral membrane protein	0.8	0.7	0.8	0.3
		HP0310	JHP0295	Conserved hypothetical protein	0.7	0.6	0.6	0.4
		HP1285	JHP1205	Conserved hypothetical secreted protein	0.6	0.4	0.4	0.5
	Purines, pyrimidines, nucleosides, and nucleotides	HP1178	JHP1104	Purine-nucleoside phosphorylase, *deoD*	0.7	0.5	0.3	0.4
	Translation	HP0382	JHP0999	Putative zinc-metalloprotease, yjr117w	0.5	0.7	0.8	0.4
	Transport and binding proteins	HP1561	JHP1469	Iron (III) ABC transporter, periplasmic iron-binding protein, *ceuE1*	0.7	0.6	0.3	0.3
		HP1562	JHP1470	Iron (III) ABC transporter, periplasmic iron-binding protein, *ceuE2*	0.7	0.3	0.3	0.3
		HP1180	JHP1106	Pyrimidine nucleoside transport protein, *nupC*	0.4	0.2	0.3	0.4
		HP0653	JHP0598	Nonheme iron-containing ferritin, *pfr*	0.8	0.5	0.2	0.2
		HP0916		Iron-regulated OMP, *frpB2*	0.7	0.3	0.4	0.3
		HP1512	JHP1405	Iron-regulated OMP, *frpB4*	0.5	0.3	0.3	0.1
	Unknown function	HP0485	JHP0437	Catalase-like protein	1.1	0.4	0.5	0.5
		HP1193		Aldo-ketoreductase, putative	0.7	0.4	0.6	0.4

[a] Data presented are relative changes (*n*-fold) from the zero-time-point starting culture. Changes (*n*-fold) for a single experiment are given. Data for the biological replicate experiment are available as supplementary information at http://falkow.stanford.edu/whatwedo/supplementarydata/ and show a similar pattern of expression for each gene.

[b] Time is measured in minutes.

[c] ND, no data.

its surface structures, those that encode protein transporters, and those involved in energy metabolism. Only a few pertinent genes within each of the induced and repressed classes will be discussed below.

Up regulated genes: urease. Included on the list of up regulated genes are six genes that are part of the urease operon. Urease synthesis is directed by a seven-gene cluster, of which *ureAB* encode the structural components of the enzyme and

ureIEFGH encode accessory genes (20, 41). Activity of the enzyme is crucial for virulence, as urease is essential for colonization and persistence in the stomach (6, 25, 28, 79). While it was previously believed that cell surface-localized urease directly created a neutral microenvironment that was conducive to bacterial survival (61), a recent study has indicated that the intracellular enzyme appears to play a more important role in AR (64). Recent work has shown that UreI acts as an inner membrane proton-gated, urea-specific channel (80). The UreI pore opens as the pH of the medium drops below 6.5 and as the cytoplasmically localized urease gains access to its urea substrate. As urease activity neutralizes the local environment, the pore closes and urea transport stops, thus providing a regulated level of enzyme activity.

Increased levels of the urease transcripts validate the ability of our microarray to identify acid-regulated components, as expression of the urease operon has previously been shown to be affected by pH (1). Akada et al. showed that levels of urease transcript were modulated by a pH-dependent posttranscriptional regulatory mechanism, as shift to an acidic pH resulted in a large increase in the levels of mRNA for each of the urease genes, even in the presence of transcriptional inhibitors. Thus, the increase shown by our microarray analysis likely reflects increased mRNA stability instead of increases in urease transcription.

Transport and binding. Another category of genes whose level of expression is increased contains those predicted to have roles as transport and binding proteins (Table 1). Notable among these are *glnQ* and *glnH*, which encode components of a glutamine ABC transporter. It has been previously shown that glutamine can be used by *H. pylori* as a basic nutrient (50). The first step of this utilization is the deamination of glutamine to glutamate, which is then further dehydrogenated to yield 2-oxoglutarate and ammonia. As mentioned previously, an increased ammonia concentration could be vital to survival of *H. pylori* under acidic conditions, as this would facilitate the maintenance of pH homeostasis within the bacterial cell and buffer the microenvironment. In support of the importance of this metabolic pathway in acidic conditions, we observed rapid increases in transcription of *gdhA*, which encodes the enzyme glutamate dehydrogenase that is required for the final catalytic step prior to ammonia production. These data suggest that increased transport of glutamine into the *H. pylori* cell and subsequent metabolic breakdown of this to produce ammonia may be important mechanisms employed by the bacteria to facilitate survival.

Another pathway involved in the production of ammonia is also up regulated in the presence of acidic pH. The enzyme cystathionine gamma-synthase is predicted to be encoded by the *metB* gene of *H. pylori* and is induced at low pH. This enzyme's primary role is the catalysis of the committing step in methionine biosynthesis (23). However, in the absence of L-cysteine, the enzyme can catalyze the production of succinate, alpha-ketobutyrate, and ammonia via a gamma elimination reaction. It is presently unclear whether L-cysteine is limiting under the exposure conditions that we tested and whether increased levels of *metB* would result in the alternate reaction described, but the potential for increased availability of ammonia seems to be a recurrent theme within the adaptation process of *H. pylori* to acid pH.

Flagellar synthesis. One of the most striking findings of our microarray analysis was the altered expression of a large number of genes that encode components of the flagellar apparatus (Table 1). *H. pylori* typically possesses a polar bundle of two to six sheathed flagella that are critical for colonization in animal models of infection (16, 26, 29, 30, 38, 58). It is believed that motility plays an essential role in that it is necessary to direct the bacterium from the extremely acidic stomach lumen to the more basic mucous layer of the gastric epithelium that is the preferred colonization site of *H. pylori* (71). Flagellar genes are divided into three classes according to their regulation by three different sigma factors. These classes include (i) σ^{54}-dependent genes that are considered to provide rod and hook functions for the flagella, (ii) σ^{28} genes that encode proteins necessary for the flagellar filament, and (iii) σ^{80}-dependent genes that comprise basal-body, biosynthetic, and chemotaxis genes. Analysis of the flagellar filament has shown that it is a copolymer of FlaA and FlaB (30, 42, 69, 72), where FlaA is the predominant subtype. FlaB is present as a minor constituent (39), but both FlaB and FlaA are necessary for full motility (35). *flaA* and *flaB* are regulated by σ^{28} and σ^{54}, respectively. In comparison to other well-studied models, the existence of two distinct flagellin subunits, present in different amounts and under the regulation of different sigma factors, is unique. It has been suggested that *H. pylori* has the ability to alter the relative level of FlaA and FlaB in response to different environmental stimuli (69, 71). By doing so, the bacterium could potentially adapt to microenvironments by alteration of the filament ultrastructure to change flagellar stiffness or flexibility to better suit the particular environment being encountered.

Our microarray analysis revealed that the overwhelming majority of pH-regulated flagellar components of *H. pylori* are class i genes. These include *flaB*, *flgK*, *flgB*, *flgE*, and *flgC*. In addition to these factors, we noted a striking increase in expression of *flgM*, which was recently shown to encode the *H. pylori* anti-σ^{28} factor (17). This anti-σ factor works by binding to σ^{28} and preventing interaction with the β-region of RNA polymerase (17), thus blocking expression of σ^{28}-dependent genes, such as *flaA*. Exposure of *H. pylori* to acid stress thus apparently results in a transcriptional switch that results in increased expression of class I genes, which presumably affects activity of the flagellum.

To determine whether exposure to acid would result in a phenotypic change in *H. pylori* motility, we conducted video microscopy of *H. pylori* cells exposed to neutral or acidic conditions as described in Materials and Methods. Analysis of bacteria exposed to neutral pH showed that only about 7% of these cells were motile and traveled at an average speed of 10.5 μm per s (Fig. 2). In contrast, cells that were shifted to an acidic pH showed 66% of the culture to be motile and traveled significantly faster than the pH 7.0 bacteria with an average speed of 24.3 μm per s (images are available as QuickTime movies at http://falkow.stanford.edu/whatwedo/supplementarydata/). Since the rotation of the *H. pylori* flagella has been shown to depend on proton motive force (56), it is likely that the bacterial cell is responding to increased concentrations of protons that are present at the acidic pH. The ability to sense the surrounding environment and alter motility could serve as a critical component of successful col-

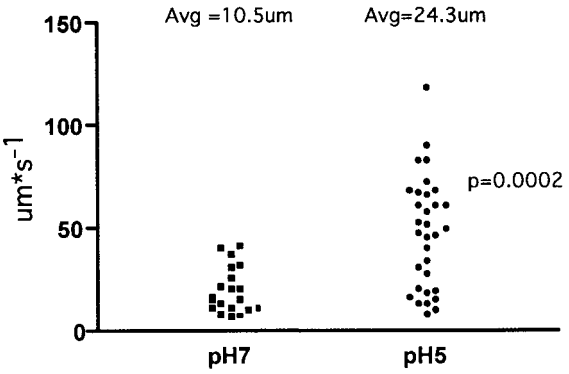

FIG. 2. The effect of acidic pH on *H. pylori* motility. A culture of *H. pylori* was split such that equal portions were suspended at pH 7.0 or 5.0. The response to the different pH conditions was monitored by video microscopy, and the speed of motile bacteria was determined as described in Materials and Methods. Each point represents one motile bacterium at the indicated pH, and the speed of that bacterium is indicated on the *y* axis. The average speed for each condition is indicated. The statistical significance of the differences in speed between the two sets of pH conditions was determined by using a two-tailed Mann-Whitney test, which revealed a *P* of 0.0002.

onization, as it would serve the purpose of directing *H. pylori* to a suitable environment for infection.

Response regulators. Another factor that exhibits increased expression upon exposure to low pH is the two-component response regulator HP1021. Within the bacterial genome of *H. pylori*, there exists a paucity of response regulatory systems, as only six genes predicted to encode response regulators and four genes predicted to encode histidine kinases are present. Previous analysis of the response regulators has shown that at least two of the five analyzed are essential for in vitro survival (8, 46), of which HP1021 is one. Little is known about the genes that are controlled by any of the regulators, with the exception of HP0166, which was recently shown to be autoregulatory and to regulate expression of a number of genes of unknown function (21). Elucidation of regulatory networks and a greater understanding of this process would potentiate knowledge about adaptive mechanisms employed by *H. pylori* that allow it to colonize within the dynamic host environ. Thus, the role of HP1021 in acid survival and elucidation of the bacterial components that it controls will be of keen interest in the future.

Down regulated genes: OMPs. Analysis of the 41 genes that reproducibly showed decreased expression between experiments also revealed that they fall into a number of different functional groups. As in the case with the induced factors, expression of a large number of hypothetical genes was affected. Somewhat surprisingly, though, this class did not represent the largest constituent of down regulated genes (Table 1). This designation instead belongs to the cell envelope classification, as 12 factors that are predicted to function as outer membrane proteins (OMPs) were identified. Comparative genomic analysis of the two sequenced *H. pylori* strains recently divided the 64 identified OMPs into five paralogous families based on sequence similarity and predicted secondary structure (4). The major outer membrane family is composed of two subfamilies; the Hop proteins and the Hor proteins. The Hop subfamily contains the largest number of OMPs and is characterized by the presence of a highly conserved N-terminal motif and conserved blocks of sequence at the C terminus (22, 27). Analysis of the list of acid-regulated OMPs revealed that 9 of the 12 were members of the Hop subfamily (Table 1).

The Hop subfamily of proteins contains several factors that are predicted to function as porins (22, 27) as well as adhesins to gastric mucosa (57). As a functional group, porins of gram-negative bacteria largely command the permeability properties of the outer membrane. They typically contain transmembrane diffusion channels that allow small molecules to diffuse across the outer membrane. The selectivity of many porins is typically based on size, though some exhibit a high degree of specificity for specific substrates (14, 43, 73). Little is known about the selectivity of most of the Hop porins. pH-regulated alteration of expression of the Hop proteins, specifically those acting as porins, would presumably affect permeability of the outer membrane and thus limit the accessibility of damaging agents to the bacterial periplasm and beyond. Precedence for the role of porins in survival upon exposure to acid pH can be found with the gastrointestinal pathogen *Vibrio cholerae*, where expression of the OmpT porin has been shown to be repressed by low pH and where the OmpU porin has been shown to be necessary for AR (51). Future experimentation to determine the selectivity of the porin proteins and the individual requirement for each in AR of *H. pylori* could shed valuable insight on the process of gastric colonization.

CagA. We did not expect to identify *cagA* as consistently repressed by low pH in our studies, as previous reports have suggested that *cagA* expression is induced at low pH (3, 37). *cagA* is part of a pathogenicity island (PAI) that encodes a novel type IV secretion apparatus in some strains of *H. pylori* (2, 15). The PAI is a 40-kilobase locus that contains at least 27 genes, and *H. pylori* strains carrying the PAI are far more likely to be associated with serious manifestations of *H. pylori* infection (81). Genes contained on the PAI show homology to genes that encode secretion machinery in other pathogenic bacteria (19), and numerous studies have shown that components of the island function in the delivery of CagA to the host cell. CagA is inserted into the plasma membrane of host cells and is phosphorylated (65) by Src-like protein tyrosine kinases (66, 70). Bacterial attachment and subsequent injection of CagA into the host cell have been shown to induce striking morphological changes in the infected cell (65), presumably as a result of binding and sequestration of the phosphatase SHP-2 (32).

To determine whether levels of CagA protein were reflective of transcript levels, we performed immunoblots by using a CagA-specific antibody on total protein harvested from *H. pylori* cells exposed to the same conditions used for our microarray analysis. As shown in Fig. 3, relative levels of CagA decreased over time and reached a level that was approximately one-half that of the starting culture, thus showing that transcriptional repression of *cagA* by acid exposure is reflected at the level of CagA accumulation. It is presently unclear why our results differ from previous reports; however, direct comparisons of the results from this and the previous studies are complicated by the different media used, lengths of exposure to acidified media, sensitivity of the assay, and different strains tested. The result may suggest that there are differences in *cagA* expression that are intricately linked to the strain being

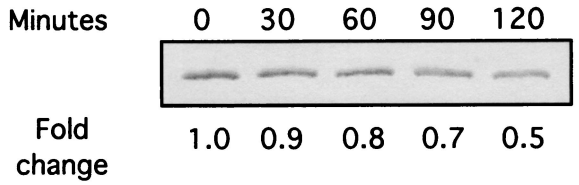

FIG. 3. The effect of low pH on CagA accumulation. Total protein was harvested from G27 shifted to pH 5.0 brucella broth plus 10% FBS at the times indicated. An equal amount of protein from each point was separated on a 6% polyacrylamide gel, transferred to nitrocellulose membranes, and probed with an anti-CagA specific antibody. The relative change (*n*-fold) from the zero time point is indicated underneath each lane.

examined. This variability could be of clinical importance due to the predicted role of CagA in disease development and progression.

Construction and characterization of an *ansB* mutant of *H. pylori*. *H. pylori* is able to generate ammonium via the actions of multiple enzymes (47). These include an aliphatic amidase (68) and at least four amino acid deaminases; asparaginase, aspartase, glutaminase, and serine dehydratase (50). The role of these amino acid deaminases in protection of *H. pylori* from acid stress is unclear, though production of intracellular ammonium could presumably facilitate survival at low pH. Our microarray analysis showed that HP0294 and HP1238, two genes predicted to encode aliphatic amidases, were rapidly induced, while HP0723, which is predicted to encode an L-asparaginase II enzyme, AnsB, was repressed (Table 1). Two major subgroupings of L-asparaginase enzymes exist: class I enzymes are constitutive cytoplasmic enzymes, while class II enzymes are secreted to the periplasm and show regulated activity (33). The action of both classes of L-asparaginase enzymes catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonium, though class II enzymes sometimes show a broader substrate specificity (33).

Previous investigations of environmental conditions that regulate gene expression have, of course, revealed both induced and repressed genes. Subsequent study of these regulated factors, however, has virtually always focused on the induced genes and ignored the repressed factors as less interesting. However, a recent report showed that in some cases induced factors are not required for survival under the conditions to which they are responding (11). Another recent study also showed that activity of an acid-repressed gene was essential for low-pH survival of *V. cholerae* (53). In that work, expression of the *recO* gene, which is required for daughter strand gap repair (reviewed in reference 40), was shown to be dramatically repressed by exposure to acid. However, activity of the protein was essential for low-pH survival, as *recO* null mutants were 1,000-fold more sensitive to acid stress than was the wild type. In addition, *vacA*, which is an acid-repressed gene (Table 1), while presumably having no role in in vitro AR, was previously shown to play an important role during infection in the murine model of *H. pylori* infection (63). For these reasons, we chose to investigate the role of the low-pH-repressed gene *ansB*, predicted to encode L-asparaginase II, in *H. pylori* acid survival and colonization.

Deletion-insertion mutations of the *ansB* coding sequence were constructed by allelic exchange in the gerbil-adapted *H. pylori* strain B128 (60), as we were unsuccessful in establishing colonization of the G27 isolate in the Mongolian gerbil model of *H. pylori*. We chose to use the gerbil model, as it has several advantages over the conventional mouse model. These include a lower gastric pH and the *H. pylori*-inducible manifestations of typical gastritis, gastric ulceration, and carcinoma that more closely mimic disease symptoms seen in humans (34). The deletion-insertion of the *ansB* coding sequence was confirmed by Southern blot analysis (data not shown). The deletion-insertion mutation in *ansB* is unlikely to exert a polar effect, since the nearest downstream gene is convergently transcribed to *ansB* and since there is a predicted rho-independent terminator immediately downstream of the *ansB* coding sequence.

Growth curve analysis of the wild-type and *ansB* strains revealed that the two strains exhibited nearly identical growth curves for the duration of the experiment (Fig. 4). Acid sensitivity assays on both strains also revealed no difference in the relative levels of AR of the two strains (data not shown). Thus, *ansB* appears to not be required for in vitro acid survival. We next hypothesized that *ansB*, though subject to acid stress regulation, perhaps plays an essential role in other stressful conditions, such as during gastric colonization. To investigate the role of *ansB* in vivo, competition assays were conducted by coinfection of the *ansB* and wild-type strains in Mongolian gerbils. Five animals were infected with a 1:1 mixture of the two strains, and a replicate of the experiment was repeated on a separate day. Animals were sacrificed after 2.5 to 3 weeks, and the surviving bacteria were collected by dissection and mechanical homogenization of the stomach. Numbers of *ansB* and wild-type bacteria were determined, and a competitive index was calculated. Strains exhibiting equal fitness for colonization would be expected to possess a competition index at or near 1, while a mutation that rendered the strain less proficient would result in a competitive index of less than 1. As shown in Fig. 5, the *ansB* mutant was attenuated in its ability to compete with the wild-type strain in both experiments and showed a geometric mean competitive index of 0.004 and 0.005, respectively. This was not the case, however, when wild-type and *ansB* strains were grown together in vitro overnight in

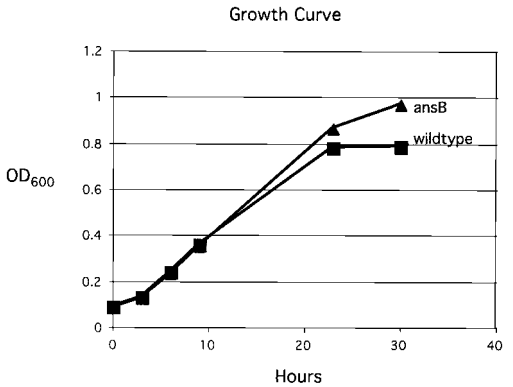

FIG. 4. Growth curve analysis of wild-type B128 and its *ansB* derivative. Brucella broth cultures supplemented with 10% FBS were started from overnight cultures of each strain. Strains were grown in microaerophilic conditions with shaking as described in Materials and Methods, and samples were taken for OD_{600} measurements at the indicated times.

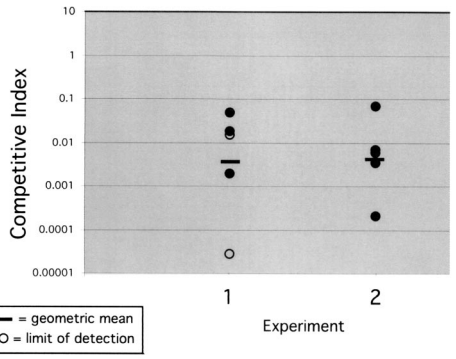

FIG. 5. *ansB* mutants of *H. pylori* are defective for in vivo competition. Equal concentrations of wild-type and *ansB* strains were mixed and were used to infect Mongolian gerbils. Colonizing bacteria were recovered after approximately 2.5 to 3 weeks, and the number of CFU of each strain was determined by differential plating. Each circle represents an infected animal, and open circles represent animals for which no *ansB* derivatives were isolated. Data for two independent experiments are depicted, and the geometric means for each (0.004 and 0.005) are indicated by a black bar.

brucella broth, as the *ansB* showed a competitive index of approximately 1.0.

Conclusion. Aformidable challenge in understanding how bacterial pathogens colonize and cause disease in their hosts is the development of a more thorough understanding of the adaptive mechanisms employed by these pathogens within the host. The development of microarray technology has provided a powerful tool for the simultaneous elucidation of the complete transcriptome of microbial pathogens, though the direct application of these to host-derived pathogens has been limited, with some notable exceptions (52, 82), by the inability to obtain a sufficiently large concentration of pure bacterial RNA from infected tissues. Due to this, researchers typically rely on the ability to mimic in vivo environments in vitro. The gastric pathogen *H. pylori* undoubtedly encounters a number of environmental stresses within the human stomach, where it colonizes. One of the most obvious factors that it must deal with is the drastic fluctuation in pH that occurs in the stomach. As such, acid-regulated gene expression is of considerable interest in understanding the process of colonization and survival of this organism.

Herein we describe a microarray-based screen to identify genes whose expression is affected upon shift to acidic pH. Our analysis revealed that 118 genes showed a twofold-or-greater change in relative expression in two independent experiments. Among these were many genes known to be regulated by low pH, such as the urease gene cluster, thus validating our ability to identify pH-regulated components. In addition, many factors whose pH regulation has not previously been recorded were identified.

Two previous array-based studies have analyzed to some extent the response of *H. pylori* to acid stress (3, 7). Both studies identified a number of factors that were pH regulated in the conditions that were tested, but there was no overlap between the genes identified in these two studies. Our study showed six genes whose expression patterns directly overlapped with these previous two array studies. These include HP1050, HP0743, HP0295, HP1440, HP0123 and HP0229. The low number of genes that overlap between the studies is probably due to differences in experimental design. In the study conducted by Allan et al. (3), the authors used nylon-based microarrays to analyze changes in gene expression that occur after a 30-min shift to pH 4.0 citrate buffer. In a single experiment, these authors found only 11 genes that showed differential expression. This number may be aberrantly low, because the microarray was constructed by using a library of pBluescript clones and thus probably does not contain a complete representation of all of the ORFs of the organism. In addition, nylon-based arrays and radioactive detection are of lower sensitivity than are the DNA-spotted glass slides and fluorophore-based strategies presently being used. The study by Ang et al. (7) utilized nylon-based arrays and biotinylated probes to examine acid-induced gene expression of *H. pylori*. Total RNA was collected from bacteria grown on acidified-agar plates for 48 h and was used to examine transcriptome profiles. The authors identified 84 genes that showed differential regulation under these conditions. Unfortunately, direct comparison of these results to ours is complicated because the bacteria were subject to a complex growth environment (colony growth), which no doubt results in very dramatic differences in the growth phases and physiological states of the bacteria examined.

It is readily accepted that a core set of genes are consistently expressed throughout the growth cycle of a bacterial cell. However, bacteria alter expression of other genes as a function of their growth phase, expressing highly divergent and specific sets of genes in the logarithmic and stationary phases. This is a well-studied paradigm in *Escherichia coli*, where transition from rapid growth to stationary phase results in the up regulation of genes that are considered to be part of the canonical RpoS or stationary-phase sigma factor regulon (reviewed in reference 31). These genes then function in diverse pathways involved in nutrient acquisition, stress survival, and alteration of the cell envelope. Accordingly, a recent analysis of growth-phase-dependent gene expression of *H. pylori* showed that, similar to what is found in other bacteria, divergent sets of genes are expressed depending on the overall growth state of the cell (76). It is possible that some of the differences in gene expression obtained by Ang et al. (7) may partially be explainable by the different growth phases of the bacteria in the two sets of conditions examined.

Future experiments to identify the role of each of the identified factors in adaptation to acidic environments should provide valuable information concerning the mechanisms employed by *H. pylori* during colonization within the gastric environment of the human host. As AR is a crucial part of *H. pylori*'s ability to survive in its human host and cause disease, study of some of these factors may facilitate elucidation of novel targets for vaccine development and antimicrobial therapy.

ACKNOWLEDGMENTS

We are grateful to D. Israel and R. Peek for providing the B128 gerbil-colonizing strain and for helpful discussion of animal experiments. A. Camilli, E. Joyce, L. Thompson, M. Amieva, and A. Mueller provided invaluable discussion and critical review of the manuscript.

D.S.M. is supported by NIH training grant AI07502-06, the Damon Runyon Cancer Research Fund, and Stanford Digestive Disease Cen-

ter grant DK56339. Research in the laboratory of S.F. and L.S.T. is supported by grants CA92229 and AI38459 from the NIH.

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Editor: V. J. DiRita