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The Changes of Proteomes Components of *Helicobacter pylori* in Response to Acid Stress without Urea

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Acid stress is the most obvious challenge *Helicobacter pylori* encounters in human stomach. The urease system is the basic process used to maintain periplasmic and cytoplasmic pH near neutrality when *H. pylori* is exposed to acidic condition. However, since the urea concentration in gastric juice is approximately 1 mM, considered possibly insufficient to ensure the survival of *H. pylori*, it is postulated that additional mechanisms of pH homeostasis may contribute to the acid adaptation in *H. pylori*. In order to identify the acid-related proteins other than the urease system we have compared the proteome profiles of *H. pylori* strain 26695 exposed to different levels of external pH (7.4, 6.0, 5.0, 4.0, 3.0, and 2.0) for 30 min in the absence of urea using 2-DE. Differentially expressed proteins were identified by MALDI-TOF-TOF-MS analysis, which turned out to be 36 different proteins. The functions of these proteins included ammonia production, molecular chaperones, energy metabolism, cell envelope, response regulator and some proteins with unknown function. SOM analysis indicated that *H. pylori* responds to acid stress through multi-mechanisms involving many proteins, which depend on the levels of acidity the cells encounter.

***Keywords*:** *Helicobacter pylori*, acid stress, comparative proteomics

Helicobacter pylori has the unique ability to survive in the extremely acidic environment of the human stomach. When migrating from acidic lumen into gastric mucus layer, *H. pylori* invariably faces pH fluctuations. The pH of human stomach content ranges from 1.0 during starvation to 5.0 in the digestive phase, though the mucus layer overlaying the gastric epithelium, where *H. pylori* colonizes, generally remains more neutral (Pflock *et al*., 2004). Therefore, *H. pylori* must sense and respond to short-term acid exposure during its infection and transmission (Young *et al*., 2000). The urease system is one of the best-studied processes of *H. pylori* in the maintenance of neutral periplasmic and cytoplasmic pH when exposed to acidic condition. This system helps to elevate the pH of the bacterial microenvironment by converting urea into ammonia and carbon dioxide (Scott *et al*., 2000; Hong *et al*., 2003). However, the urea in gastric juice is approximately 1 mM, which sometimes may be insufficient to ensure the survival of *H. pylori* (Wen *et al*., 2003). Thus it is assumed that additional mechanisms of pH homeostasis may be required for the acid adaptation in *H. pylori*.

Genomic and proteomic technologies, despite their apparent differences, are both integrative and high-throughput technologies whose rapid development has led to a burst across all branches of the life sciences (Iii and Osterman, 2007). To explore other possible mechanisms of *H. pylori* response to acid stress, many researchers have investigated the acid-related genes under different acidic conditions utilizing DNA microarrays (Allan *et al*., 2001; Ang *et al*., 2001; Merrell *et al*., 2003; Wen *et al*., 2003). However, proteins are the direct executors of vital activities and mRNA level is not consistently reliable to predict protein abundance due to the posttranslational and posttranscriptional modification (Hegde *et al*., 2003). Furthermore, proteomic technologies have been proven to be particularly useful to study the physiological responses of bacteria to various environmental stresses (Brotz-Oesterhelt *et al*., 2005). Slonczewski *et al*. (2000) have studied the protein profile of *H. pylori* growing in media buffered at pH 7.5 and 5.7 using proteomic technologies but the pH they chose could not represent the pH range of the human stomach where *H. pylori* colonizes. Additionally, Mizogubhi *et al*. (1998) has studied protein changes in coccoid forms of *H. pylori* under pH 7.4, 5.0, 3.5, and 2.0 for 1 h using one-dimensional SDS-PAGE, which showed that acid shock, even for a period of 1 h, did not inhibit the production of proteins but, conversely, induced the synthesis of some proteins.

Although *H. pylori* has been extensively studied for its sensitivity and tolerance to acid stresses, its response to acidity still remains largely uncharacterized. In this study, we examined the changes in protein profiles of *H. pylori* under short-term acid exposure to pH 2.0, 3.0, 4.0, 5.0, and 6.0 in the absence of urea, in an attempt to discover new acid-adaptive molecules in this bacterium.

Materials and Methods

Bacterial strain and culture conditions

H. pylori strain 26695 was kindly provided by Dr. Zhang Jianzhong in the Chinese Disease Control and Prevention

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Center. The bacteria were cultured in Brucella broth containing 10% fetal bovine serum with 120 rpm shaking in microaerobic environment (5% O_2, 10% CO_2, and 85% N_2) at 37°C. Then the same amount of exponentially growing *H. pylori* was resuspended in urea-free RPMI 1640 medium with pH 2.0, 3.0, 4.0, 5.0, 6.0, and 7.4 adjusted by concentrated HCl after sterilization. The cultures were incubated under microaerobic conditions at 37°C for 30 min.

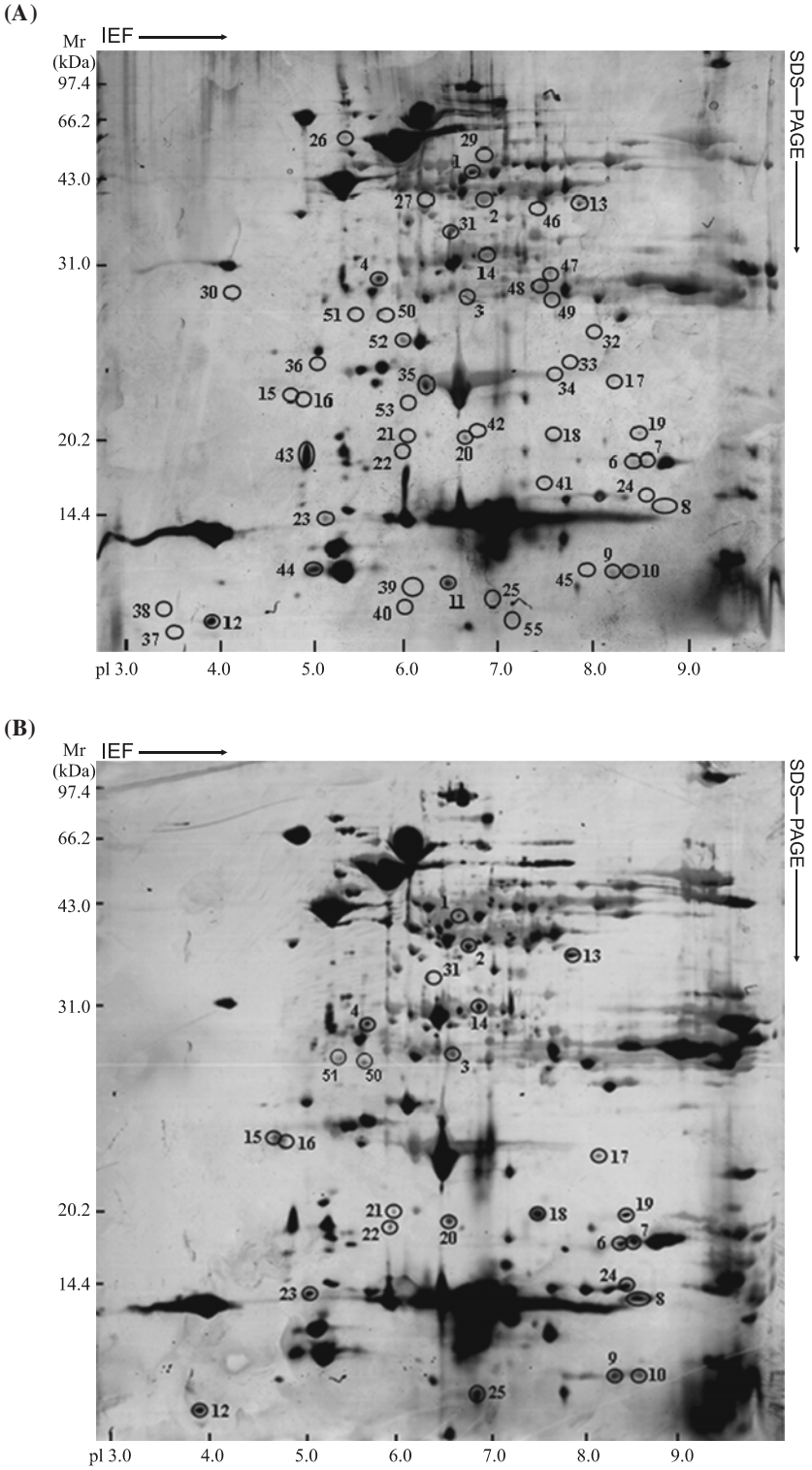

Fig. 1. Representative 2-DE maps of whole cell proteins from *H. pylori* 26695 exposed to pH 7.4 and 4.0 for 30 min in the absence of urea. (A) *H. pylori* grown in medium with pH 7.4 and (B) *H. pylori* exposed to pH 4.0 for 30 min.

2-DE and image analysis

Bacterial cells exposed to pH 7.4, 6.0, 5.0, 4.0, 3.0, and 2.0 for 30 min were harvested by centrifugation at 5,000×g for 10 min at 4°C, washed three times with ice-cold PBS (pH 7.2) and solubilized in lysis buffer containing 8 M urea, 2 M thiourea, 4% CHAPS, 1% DTT, 1% pharmalyte (pH range 3∼10), 1% protease inhibitor, and 1% nuclease mix (Amersham biosciences). After sonication, the solution was centrifugated at 20,000×g for 60 min at 4°C. Insoluble material was discarded. The protein concentration was measured by the Bradford method and proteins were stored at -80°C until 2-DE analysis.

For the first-dimensional separation, isoelectric focusing (IEF) was carried out using 18 cm IPG strips (pH 3∼10, NL) and an IPGphor instrument (Amersham biosciences). One hundred microgram of the total proteins were diluted to a total volume of 340 μl with rehydration solution containing 8 M urea, 4% CHAPS, 20 mM DTT, 0.5% pharmalyte and a trace of bromophenol blue. The strip was rehydrated for 12 h at 60 V. The voltage was linearly increased from 100 V to 8,000 V during the first 4 h and then maintained constant at 8,000 V to a total of 80 kVh. After IEF, the IPG strips were equilibrated for 15 min each in the equilibration solution (50 mM, Tris-HCl; pH 8.8, 6 M urea, 30% glycerol, 2% SDS, a trace of bromophenol blue) with 0.5% (w/v) DTT and 2% (w/v) iodoacetamide respectively under gentle shaking. In the second dimension, IEF strips were placed on top of a 13% continuous vertical SDS-PAGE gel and covered by 0.5% agarose gel. SDS-PAGE was conducted at 15 mA per gel for 15 min, and then 30 mA per gel until the bromophenol blue front reached bottom of the gel using the PROTEANII xi 2-D cell (Bio-Rad). The gels were then silver-stained, and the protein patterns in the gels were scanned using ImageScanner (Amersham biosciences) at the 256 grayscale and 300 dpi degree level. 2-DE was repeated three times using independently grown cultures. The gel of *H. pylori* grown in medium with pH 7.4 was used as the reference. Other gels were matched with it using ImageMaster 2D Elite 5.0 (Amersham biosciences) to find differentially expressed protein spots.

In-gel digestion and MALDI-TOF-TOF MS

Differentially expressed protein spots were excised, tryptic digested and identified with a 4700 MALDI-TOF-TOF proteomic system (Applied Biosystems) as the protocols described previously (Yan *et al*., 2006). The MS and MS/MS spectra were analyzed with a 50 ppm mass tolerance by GPS Explorer V.2.0.1 and Mascot V1.9 based on NCBI SWISSPROT and local *H. pylori* databases (April, 2006 updated). Background peaks from known trypsin auto-digestion fragments and common contaminants were automatically excluded prior to database search. Oxidation of methionine and carbamidomethylation of cysteines were allowed as variable modifications. Identifications with a GPS confidence interval of greater than 99% were accepted.

Self-organizing map (SOM) analysis

In order to observe the dynamic protein regulation pattern in *H. pylori* exposed to various external pH in the present study, the logarithm based on 2 of %vol ratio of all identified altered proteins between samples and control were subjected to (Self-organizing map) SOM analysis performed as described previously (Zheng *et al*., 2005). Illustration of SOM output was conducted in MATLAB6.5 environment (www.mathworks.com).

Results and Discussion

In order to colonize and propagate in extremely acidic stomach, *H. pylori* not only possesses the ability to tolerate and resist acid stress but also develops an acid-adaptive response. To imitate the short-term acid exposure during its infection, we suspended exponentially growing *H. pylori* in medium of pH 2.0, 3.0, 4.0, 5.0, and 6.0 for 30 min, which were all within the physiological gastric pH range. We did not add urea because previous studies have shown that urease could be used to elevate the periplasmic pH within several minutes, which makes it hard to detect urease-independent mechanisms (Toledo *et al*., 2002; Wen *et al*., 2003).

Protein profiles of *H. pylori* under acid stress

Proteomic analysis allowed identification of 36 proteins whose expression changed (more than twofold, $P<0.05$) in response to acid (Fig. 1). Among them, 26 proteins were induced and the other 10 proteins were repressed under acidic conditions. A summary of the main proteins is shown in Table 1. These proteins are involved in many cellular functions including ammonia production, molecular chaperones, energy metabolism, cell envelope, response regulation as well as some unknown functions (Fig. 2). Remarkably, urease and aliphatic amidase that have been previously shown to be closely related to acid stress were among these proteins, which validated our approach to identify acid-related proteins.

Urease is central to the pathogenesis of *H. pylori* and essential for its survival in acidic environment both *in vivo* and *in vitro*. In this study, urease alpha subunit (*ureA*), urease beta subunit (*ureB*) and urease accessory protein (*ureG*) were upregulated by acid stress even though urea was not added to the medium. The upregulation of *ureA* and *ureB* at pH 5.0 is not surprising since they are likely co-transcribed from the same promotor and are both essential for the activity of urease (McGee *et al*., 1999). Aliphatic amidase (*amiE*) is another ammonia-producing enzyme of *H. pylori,* which hydrolyzes short-chain amides to produce ammonia and the corresponding organic acid. The acquisition of this enzyme might give the strain a metabolic advantage when colonizing an unusual ecological niche such as the stomach (Bury-Mone *et al*., 2003). The induction of this enzyme plays a role in pH homeostasis under urea deficient acidic condition.

One mechanism of *H. pylori* to regulate cellular proton level is restricting the acute entry of protons across its membrane and extruding H^+ from the cell. ATP synthase catalyzes the synthesis of ATP from ADP and Pi, utilizing energy derived from proton electrochemical gradient formed by electron transport (Hazard and Senior, 1994). Our results showed that ATP synthase F1, subunit delta (*atpH*), and subunit alpha (*atpA*) upregulated at acidic condition. The induced expression of this enzyme contributes to the pH homeostasis by extruding H^+ from *H. pylori*. The study by

Table 1. Summary of primary proteins showing altered expression under acid stress

Function class	Spot no.[a]	Proteins (genes)	TIGR ORF no.[b]	Top score	Sequence coverage (%)	%vol ratio (pH)[c]				
						6.0	5.0	4.0	3.0	2.0
Molecular chaperones	3	co-chaperone and heat shock protein (*groES*)	Hp0011	84	46	5.86	4.67	2.42	1.65	5.26
	9	Thioredoxin(*trxA*)	Hp0824	151	93	3.03	4.53	2.19	1.21	1.84
	10	Thioredoxin(*trxA*)	Hp0824	162	96	2.59	2.08	2.10	0.87	1.52
	31	thioredoxin reductase (*trxB*)	Hp0825	177	74	0.09	0.35	0.03	0.07	0.01
	51	chaperone and heat shock protein (*groEL*)	Hp0010	59	32	1.53	1.80	2.26	2.16	1.63
Ammonia production	2	aliphatic amidase (*amiE*)	Hp0294	292	76	1.27	1.83	2.02	0.92	0.39
	36	urease accessory protein (*ureG*)	Hp0068	88	44	1.08	1.44	17.1	1.50	1.67
	47	urease alpha subunit (*ureA*)	Hp0073	85	29	0.91	2.01	2.23	0.87	0.44
	51	urease beta subunit (*ureB*)	Hp0072	70	33	1.53	1.98	2.26	1.76	1.63
Response regulator	50	response regulator (*ompR*)	Hp0166	81	41	1.35	1.82	2.13	1.52	2.06
Virulence	20	cag pathogenicity island protein (*cag24*)	Hp0545	73	39	0.92	0.96	2.00	0.27	0.89
Cell envelope	33	outer membrane protein	Hp1564	213	61	0.88	0.96	0.58	6.11	1.26
Metabolism	1	beta ketoacyl-acyl carrier protein synthase II (*fabF*)	Hp0558	163	70	0.50	0.47	0.17	0.52	0.68
	4	acetyl-coenzyme A carboxylase (*accA*)	Hp0557	60	37	0.60	0.21	0.49	0.82	0.46
	4	fumarate reductase, iron-sulfur subunit (*frdB*)	Hp0191	127	64	0.60	0.21	0.49	0.82	0.46
	13	aspartate-semialdehyde dehydrogenase (*asd*)	Hp1189	338	65	4.09	0.76	2.05	1.52	1.27
	14	malonyl coenzyme A-acyl carrier protein transacylase (*fabD*)	Hp0090	189	55	2.03	0.87	2.14	1.23	1.75
	17	ATP synthase F1, subunit delta (*atpH*)	Hp1135	257	91	2.34	1.24	2.13	1.05	1.75
	31	fructose-bisphosphate aldolase (*tsr*)	Hp0176	61	32	0.09	0.35	0.03	0.07	0.01
	26	ATP synthase F1, subunit alpha (*atpA*)	Hp1124	79	26	1.11	2.50	1.49	28.1	1.09
Replication and translation	19	ribosome releasing factor (*frr*)	Hp1256	118	55	1.75	1.84	2.83	1.48	1.59
	21	neutrophil activating protein (*napA*)	Hp0243	82	62	3.29	3.78	2.82	0.91	2.23
	22	neutrophil activating protein (*napA*)	Hp0243	96	72	1.36	2.26	2.86	0.84	0.88
	26	translation elongation factor EF-Tu (*tufB*)	Hp1205	105	41	1.11	2.50	1.49	28.1	1.09
Unknown function	6	hypothetical protein	Hp0721	205	44	2.12	1.77	2.16	0.70	0.38
	7	phenylacrylic acid decarboxylase	Hp1476	48	46	3.47	2.17	3.39	1.10	0.45

[a] Spot numbers refer to the proteins labeled in Fig. 1.
[b] TIGR ORF no. follows the nomenclature of *H. pylori* strain 26695
[c] %vol ratio for each protein derived from *H. pylori* cultured at pH 6.0, 5.0, 4.0, 3.0, 2.0 with respect to the protein derived from *H. pylori* at pH 7.4.

Bijlsma *et al*. (2000) also indicates that F1F0 ATPase is essential for the growth of *H. pylori* under acidic conditions. Moreover, we found one outer membrane protein induced and three proteins involved in fatty acid and phospholipids metabolism affected by low pH. They are beta ketoacyl-acyl carrier protein synthase II (fabF), acetyl-coenzyme A carboxylase (accA) and malonyl coenzyme A-acyl carrier protein transacylase (fabD). The changes of these proteins may contribute to the permeability of the outer membrane and maintenance of a favorable periplasmic and intracellular pH of bacteria. In addition, we also found fumarate reductase and fructose-bisphosphate aldolase repressed under acid stress. They are important enzymes in carbohydrate metabolism. It appears that *H. pylori* may respond to low pH by carbohydrate metabolic buffering, which is consistent with previous reports in other bacteria (Yohannes *et al*., 2004;

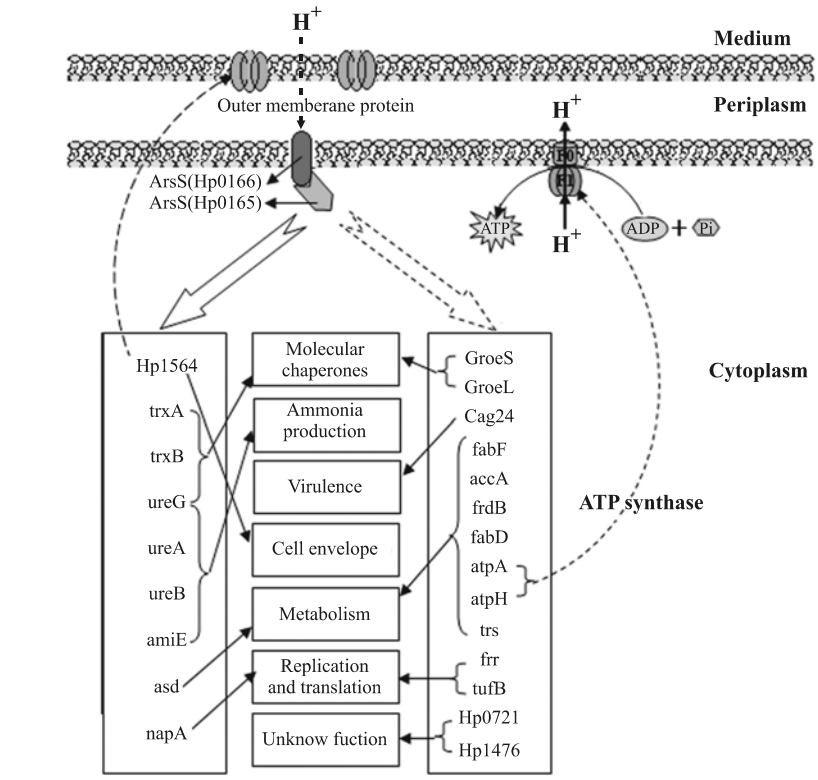

Fig. 2. A simple model of acid stress response in *H. pylori* under condition lacking urea. *H. pylori* can perceive signals of low pH by ArsS or other putative sensors and transmit them to the cellular machinery by signal transduction. ArsR can regulate expression of some proteins (solid arrow). Other proteins (dash arrow) have not been proved to be regulated by ArsR. *H. pylori* can establish a new pH homeostasis by changing the abundance and activities of functional proteins involved in molecular chaperones, ammonia production, cell envelope, and metabolism.

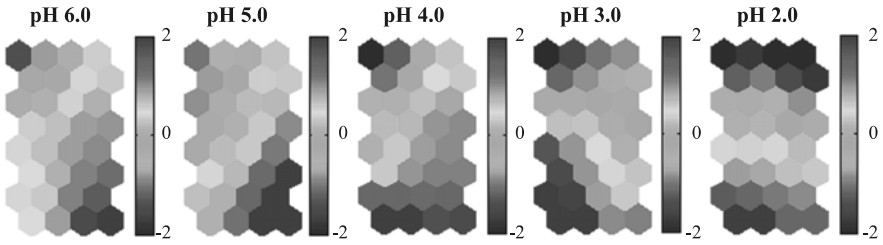

Fig. 3. Visualization of the SOM outputs of dynamic proteome changes underlying *H. pylori* exposed to different level of external pH (6.0, 5.0, 4.0, 3.0, 2.0) for 30 min in the absence of urea. The logarithm based on 2 of %vol ratio of all identified altered proteins between samples and control were subjected to (Self-organizing map) SOM analysis performed as described previously. Each presentation illustrates a pH-specific proteomic map of identified proteins in present study. All up-regulated, down-regulated, and moderately regulated proteins are well delineated.

Kurian *et al*., 2006).

Two-component systems exist widely in prokaryotes and regulate their responses to changing environmental conditions. *H. pylori* is one of the organisms with the smallest number of two-component systems whose genome sequences have been characterized. In the present study, we detected one response regulator (Hp0166) induced under acidic condition. The study by Wen *et al*. (2003) about acid stress on *H. pylori* showed that Hp0166 was up-regulated by acidity at transcriptional level. Several studies also observed a number of genes that were regulated by Hp0165-0166 two-component system (Bury-Moné *et al*., 2004; Wen *et al*., 2006; Scott *et al*., 2007). Our study is the first report that the expression of Hp0166 is changed under acidic condition at the protein level. This further verifies its important role in the acid adaptive response of *H. pylori* again.

In the present study, we found some other proteins whose expression changed during pH shift. Previously, heat shock proteins have been observed to be upregulated in *H. pylori* under acid stress (Allan *et al*., 2001). Our present study also demonstrated that heat shock protein GroES/GroEL were induced by acid stress. It has been proposed

that they play important roles in the adaptation of *H. pylori* to acid environment by regulating the expression of urease (Suerbaum *et al*., 1994; Huesca *et al*., 1998). The thioredoxin system behaves as a stress response element in *H. pylori* and protects it from damage by initiating the process of catalytic reduction of susceptible target proteins (Windle *et al*., 2000; Comtois *et al*., 2003). The up-regulation of thioredoxin in our study indicates that it plays an important role in *H. pylori* under acid stress. Ribosome releasing factor and translation elongation factor EF-Tu related to translation, as well as several proteins with so-far unknown functions such as Hp0721 have also shown differential expression under acidic condition.

SOM analysis

As shown in Fig. 3, the SOM outputs offered a global view of the identified protein expression profiles in *H. pylori* after various pH exposures for 30 min. It can be seen that proteins mapped to the corner/edge areas of the Fig. are mostly regulated under different acidic condition. The results indicated that the expression of more proteins was inhibited with the decrease of pH. At pH 6.0, several proteins at the left-upper corner were shown to be downregulated. These proteins may be the adaptive proteins of *H. pylori* responding to weak acid stress. We can see that the inhibited proteins extended from the left-upper corner to the right-upper corner with the decreasing of pH. In addition, the upregulated proteins at acidic condition showed different pattern. Several proteins at the left-lower corner that were markedly regulated at weak acidic condition (pH 6.0 and 5.0) showed only slight upregulation at strong acidic condition (pH 3.0 and 2.0). On the other hand, proteins slightly regulated at weak acidic condition were significantly upregulated at strong acidic condition. This indicated that the adaptive mechanisms of *H. pylori* display notable distinction at different acidic condition. The map at pH 4.0 seems to be a transitional regulation pattern. All of above suggested that *H. pylori* responds to acid stress through multi-mechanisms involving many signals, which depend on the levels of acidity the cells encounter.

In summary, we have identified 36 proteins in *H. pylori* that are involved in various cellular functions during response to acidic condition through proteomic analysis. SOM analysis indicated that the adaptive mechanisms of *H. pylori* display notable distinction at different acidic condition. These findings provide more information to better understand how *H. pylori* survives in the acidic environment of the human stomach.

Acknowledgements

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References

Allan, E., C.L. Clayton, A. McLaren, D.M. Wallace, and B.W. Wren. 2001. Characterization of the low-pH responses of *Helicobacter pylori* using genomic DNA arrays. *Microbiology* 147, 2285-2292.

Ang, S., C.Z. Lee, K. Peck, M. Sindici, U. Matrubutham, M.A. Gleeson, and J.T. Wang. 2001. Acid-induced gene expression in *Helicobacter pylori*: study in genomic scale by microarray. *Infect. Immun.* 69, 1679-1686.

Bijlsma, J.J., M. Lie-A-Ling, I.C. Nootenboom, C.M. Vandenbroucke-Grauls, and J.G. Kusters. 2000. Identification of loci essential for the growth of *Helicobacter pylori* under acidic conditions. *J. Infect. Dis.* 182, 1566-1569.

Brotz-Oesterhelt, H., J.E. Bandow, and H. Labischinski. 2005. Bacterial proteomics and its role in antibacterial drug discovery. *Mass Spectrom. Rev.* 24, 549-565.

Bury-Mone, S., S. Skouloubris, C. Dauga, J.M. Thiberge, D. Dailidiene, D.E. Berg, A. Labigne, and H. De Reuse. 2003. Presence of active aliphatic amidases in *Helicobacter* species able to colonize the stomach. *Infect. Immun.* 71, 5613-5622.

Bury-Moné, S., J.M. Thiberge, M. Contreras, A. Maitournam, A. Labigne, and H. De Reuse. 2004. Responsiveness to acidity via metal ion regulators mediates virulence in the gastric pathogen *Helicobacter pylori. Mol. Microbiol.* 53, 623-638.

Comtois, S.L., M.D. Gidley, and D.J. Kelly. 2003. Role of the thioredoxin system and the thiol-peroxidases Tpx and Bcp in mediating resistance to oxidative and nitrosative stress in *Helicobacter pylori. Microbiology* 149, 121-129.

Hazard, A.L. and A.E. Senior. 1994. Mutagenesis of subunit delta from *Escherichia coli* F1F0-ATP synthase. *J. Biol. Chem.* 269, 418-426.

Hegde, P.S., I.R. White, and C. Debouck. 2003. Interplay of transcriptomics and proteomics. *Curr. Opin. Biotechnol.* 14, 647-651.

Hong, W., K. Sano, S. Morimatsu, D.R. Scott, D.L. Weeks, G. Sachs, T. Goto, S. Mohan, F. Harada, N. Nakajima, and T. Nakano. 2003. Medium pH-dependent redistribution of the urease of *Helicobacter pylori. J. Med. Microbiol.* 52, 211-216.

Huesca, M., A. Goodwin, A. Bhagwansingh, P. Hoffman, and C.A. Lingwood. 1998. Characterization of an acidic-pH-inducible stress protein (hsp70), a putative sulfatide binding adhesin, from *Helicobacter pylori. Infect. Immun.* 66, 4061-4067.

Iii, J.R. and A.L. Osterman. 2007. Introduction: advances in genomics and proteomics. *Chem. Rev.* 107, 3363-3366.

Kurian, D., K. Phadwal, and P. Maenpaa. 2006. Proteomic characterization of acid stress response in *Synechocystis* sp. PCC 6803. *Proteomics* 6, 3614-3624.

McGee, D.J., C.A. May, R.M. Garner, J.M. Himpsl, and H.L. Mobley. 1999. Isolation of *Helicobacter pylori* genes that modulate urease activity. *J. Bacteriol.* 181, 2477-2484.

Merrell, D.S., M.L. Goodrich, G. Otto, L.S. Tompkins, and S. Falkow. 2003. pH-Regulated gene expression of the gastric pathogen *Helicobacter pylori*. *Infect. Immun.* 71, 3529-3539.

Mizoguchi, H., T. Fujioka, K. Kishi, A. Nishizono, R. Kodama, and M. Nasu. 1998. Diversity in protein synthesis and viability of *Helicobacter pylori* coccoid forms in response to various stimuli. *Infect. Immun.* 66, 5555-5560.

Pflock, M., P. Dietz, J. Schar, and D. Beier. 2004. Genetic evidence for histidine kinase HP165 being an acid sensor of *Helicobacter pylori. FEMS Microbiol. Lett.* 234, 51-61.

Scott, D.R., E.A. Marcus, D.L. Weeks, A. Lee, K. Melchers, and G. Sachs. 2000. Expression of the *Helicobacter pylori ureI* gene is required for acidic pH activation of cytoplasmic urease. *Infect. Immun.* 68, 470-477.

Scott, D.R., E.A. Marcus, Y. Wen, J. Oh, and G. Sachs. 2007. Gene expression *in vivo* shows that *Helicobacter pylori* colo-

nizes an acidic niche on the gastric surface. *Proc. Natl. Acad. Sci. USA* 104, 7235-7240.

Slonczewski, J.L., D.J. McGee, J. Phillips, C. Kirkpatrick, and H.L. Mobley. 2000. pH-Dependent protein profiles of *Helicobacter pylori* analyzed by two-dimensional gels. *Helicobacter* 5, 240-247.

Suerbaum, S., J.M. Thiberge, I. Kansau, R.L. Ferrero, and A. Labigne. 1994. *Helicobacter pylori* hspA-hspB heat-shock gene cluster: nucleotide sequence, expression, putative function and immunogenicity. *Mol. Microbiol*. 14, 959-974.

Toledo, H., M. Valenzuela, A. Rivas, and C.A. Jerez. 2002. Acid stress response in *Helicobacter pylori. FEMS Microbiol. Lett.* 213, 67-72.

Wen, Y., J. Feng, D.R. Scott, E.A. Marcus, and G. Sachs. 2006. Involvement of the HP0165-HP0166 two-component system in expression of some acidic-pH-upregulated genes of *Helicobacter pylori. J. Bacteriol.* 188, 1750-1761.

Wen, Y., E.A. Marcus, U. Matrubutham, M.A. Gleeson, D.R. Scott, and G. Sachs. 2003. Acid-adaptive genes of *Helicobacter pylori. Infect. Immun.* 71, 5921-5939.

Windle, H.J., A. Fox, D. Ni Eidhin, and D. Kelleher. 2000. The thioredoxin system of *Helicobacter pylori*. *J. Biol. Chem*. 275, 5081-5089.

Yan, S.P., Q.Y. Zhang, Z.C. Tang, W.A. Su, and W.N. Sun. 2006. Comparative proteomic analysis provides new insights into chilling stress responses in rice. *Mol. Cell. Proteomics* 5, 484-496.

Yohannes, E., D.M. Barnhart, and J.L. Slonczewski. 2004. pH-Dependent catabolic protein expression during anaerobic growth of *Escherichia coli* K-12. *J. Bacteriol.* 186, 192-199.

Young, K.A., Y. Akyon, D.S. Rampton, S.G. Barton, R.P. Allaker, J.M. Hardie, and R.A. Feldman. 2000. Quantitative culture of *Helicobacter pylori* from gastric juice: the potential for transmission. *J. Med. Microbiol.* 49, 343-347.

Zheng, P.Z., K.K. Wang, Q.Y. Zhang, Q.H. Huang, Y.Z. Du, Q.H. Zhang, D.K. Xiao, S.H. Shen, S. Imbeaud, E. Eveno, C.J. Zhao, Y.L. Chen, H.Y. Fan, S. Waxman, C. Auffray, G. Jin, S.J. Chen, Z. Chen, and J. Zhang. 2005. Systems analysis of transcriptome and proteome in retinoic acid/arsenic trioxide-induced cell differentiation/apoptosis of promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA* 24, 7653-7658.