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Supplemental Data

Differential Proteomics of *Helicobacter pylori* Associated with Autoimmune Atrophic Gastritis

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Supplementary Table S1. List of papers about *Helicobacter pylori* proteomics and major findings since year 2000.

Strains (pathologies)	Proteomic approach	Major objectives and findings	Proteins	References
Strains including TK1402	Isolation of OMVs, 1-DE, N-terminal amino acid sequence.	To characterize the OMVs produced by strain TK1402. The 22-kDa protein may be involved in biofilm formation.	Finding of a unique 22-kDa protein in TK1402 OMVs.	(81)
Strain 26695	2-DE/MS and 1-DE-LC/MS; cytoplasmic/ membrane proteins solubilization by urea/SDS.	To create a protein database for HP. Identification of 567 proteins from 5 experiments, corresponding to 36.6% of the predicted complete HP proteome.	All the identified proteins are stored in the Proteome Database System for Microbial Research.	(82)
Strain B18 (non-cancerogenic) and 7.13 (cancerogenic)	DIGE, DNA sequencing, WB, MALDI TOF MS/MS.	To define proteins mediating the development of HP-induced gastric cancer, by identifying differentially abundant membrane and cytosolic proteins.	26 proteins significantly different between the two strains, including a novel cysteine-to-arginine mutation in the flagellar protein FlaA.	(31)
Strain 26695	Protein fractionation with stepwise concentrations of ammonium sulfate, 2-DE.	Analyses of proteome profile of HP whole-cell extracts and protein fractions by stepwise ammonium sulfate precipitation. Identification of 98 proteins by PMF, of which 29 were newly identified.	37 proteins, including: KdsA; GroEL; UreA; UreB; TrxA; NapA; FldA; NapA; SodB; CeuE, and Pfr.	(75)
Strain J99	2D BN/SDS-PAGE, LC Ion Trap MS/MS.	Analyses of both cytoplasmic and membrane multiprotein complexes. 34 different proteins were identified, which were grouped in 13 multiprotein complexes.	Description of interactions involving known pathogenic factors such as: (i) urease with the heat shock protein GroEL or the putative ketol-acid reductoisomerase IlvC and (ii) the cag pathogenicity island (PAI) CagA protein with the DNA gyrase GyrA, as well as some partners of TsaA, and a peroxide reductase/stress-dependent molecular chaperone.	(53)
Strains 26695, P12 and B128	Protein fractionations into soluble and structure/ membrane-bound, 2-DE and MALDI MS.	To establish a dynamic 2-DE database with multiple HP subproteomes. The 50 most abundant protein spots in each fraction were identified by PMF.	4 cag PAI proteins; numerous OMPs; the vacuolating cytotoxin VacA; other potential virulence factors, and few ribosomal proteins were detected in the structure-bound fraction. In contrast; catalase KatA; γ-glutamyltranspeptidase Ggt, and the neutrophil-activating protein NapA were found almost exclusively in the soluble protein fraction.	(41)

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Supplementary Table S1. ***Continued.***

Strain 26695	Isolation of a sarcosine-insoluble outer membrane fraction, 2-DE, MALDI MS and WB.	To identify the HP OMPs. A total of 62 spots, representing 35 genes, were identified together with 9 immunogenic ones.	Isoforms of phosphoglycerate dehydrogenase SerA; 16 OMPs; urease β subunit ureB; catalase; ABC transporters of iron(III); glutamine and amino acids; flagellar basal-body L-ring protein FlgH; alkyl hydroperoxide reductase TsaA; glutamine synthetase GlnA; (3R)-Hydroxymyristoyl-(acyl carrier protein) dehydratase FabZ; neutrophil-activating protein NapA.	(76)
Strain 26695	2-DE and MALDI MS.	To investigate HP proteins regulated by a Fe^{2+}-dependent transcriptional repressor in response to iron. 93 spots were found to be up- or down-regulated, of which 39 were identified.	Identification of 29 different proteins of diverse functions (e.g. energy metabolism, transcription and translation, detoxification, biosynthesis of amino acids and nucleotides and production of the cell envelope).	(60)
Strains 119/95, 32, 33 CCUG 17874 and CCUG 17875	ProteinChip® arrays coupled with SELDI TOF MS of cell surface extracts.	To establish the use of ProteinChip® technology comparing outer membrane protein profiles between fresh HP clinical isolates vs. strains heavily passaged *in vitro*, and to compare profiles between *Helicobacter* spp.	A rapid and accurate assessment of protein profiles from different *Helicobacter* spp. in particular of low MW proteins.	(83)
Strain 26695	2DE, MALDI TOF MS/MS, MS-Screener and cluster analyses.	To increase the number of HP identified proteins by automated spot picking/digestion/peak detection/database search, and to go inside the antigenic properties linked to the dimerization of an alkyl hydroperoxide reductase, the degradation of GroEL, and the fragmentation of γ-glutamyltranspeptidase.	Automatic processing of 384 spots in 3 replicates, resulting in datasets of 960 PMF; presence of some antigenic proteins in more spots: GroEL; alkyl hydroperoxide reductase, and γ-glutamyltranspeptidase in 15, 8 and 2 different spots, respectively.	(80)
Strain 26695 and J99	2-DE, MALDI TOF MS, LC ESI MS/MS of secreted proteins.	To optimize HP culture conditions for minimal autolysis and to characterize HP secretome in order to propose some potential targets for therapy and vaccine development. Resolution of HP secreted proteins into 33 spots, of which 26 were identified.	Among the secreted proteins were: several redox-active enzymes; various components of the flagellar apparatus; 6 putative oxidoreductase; 3 fragments of the vacuolating toxin VacA; the serine protease and chaperone HtrA, and 8 previously uncharacterized proteins.	(79)
Strain 26695	Selective biotinylation of intact HP and purification of labeled proteins by membrane isolation, solubilization, affinity chromatography, 2-DE.	To identify surface proteins that may play a role in pathogen-host interactions and potential targets for the control of HP infection. Separation of 82 biotinylated proteins, of which 18 were identified.	9 proteins previously shown to be surface-exposed; 7 proteins virulence-associated, and 11 highly immunogenic in infected patients.	(78)
Strain 26695	2-DE, MALDI TOF MS.	To perform an extensive proteome analysis and to construct a master protein map of the HP reference strain. Among 345 spots processed, 175 proteins were identified and 115 were newly identified.	60 proteins, such as urease-subunit; 60 kDa chaperonin, and thioredoxin. New proteins identified included type I restriction enzyme R protein; type IIS enzyme R; M protein, and DNA polymerase III-subunit.	(77)
Strain NCTC 11637	2-DE, MALDI TOF MS.	To characterize the NCTC 11637 proteome. 93 of the most intensely stained protein spots were identified, and searched for post-translational modifications.	The 93 selected proteins corresponded to 35 genes, an high degree of modifications being included in: 26-kDa antigen tsaA (12 protein spots); urease α and β subunits ureA (11 spots) and UreB (5 spots); non-heme containing ferritin pfr (8 spots) and 60 kDa chaperonin GroEL hspB (6 spots).	(42)

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Supplementary Table S1. ***Continued.***

Strains 26695, SS1 and J99	2-DE, MALDI TOF MS.	To systematically analyze HP proteome. Up to 1800 proteins separated, of which of 152 proteins identified and organized in a 2-DE database accessible via the Internet.	The 3 strains highly genetically differed. The identified proteins included: 28 antigens and 9 known virulence factors (CagA, VacA, urease α and β subunit , GroEL, flagellin, p35, and a 26 kDa antigen).	(43)
Strain17875	Liquid-phase IEF, 1-DE, MALDI TOF MS.	To identify and characterize the cell-surface proteins expressed by HP in order to further develop vaccines. 40 proteins from a detergent-solubilized HP preparation were identified, and over one-third were membrane or membrane-associated.	The Leb-binding adhesin, a functionally characterized membrane-associated protein, was found among the 40 identified proteins.	(84)

Abbreviations: 1-DE, one dimensional electrophoresis; 2-DE, two dimensional electrophoresis; BN, blue native; DIGE, differential in gel electrophoresis; DU, duodenal ulcer; G, gastritis; GC, gastric cancer; HP, *Helicobacter pylori*; IEF, isoelectric focusing; LC, liquid chromatography; MALDI, matrix-assisted laser desorption ionization; MS, mass spectrometry; MW, molecular weight; OMVs, outer membrane vesicles; PMF, peptide mass fringerprinting; PU, peptic ulcer; Q-TOF, quadrupole time of flight; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SELDI, surface-enhanced laser desorption/ionization; TOF, time of flight; WB, western blotting.

Supplementary Table S2. List of papers about *Helicobacter pylori* proteomics associated with gastric diseases since year 2000.

Pathology (HP strain, nr of pts)[a)]	Proteomic approach	Differentially expressed proteins	References
G (HS65, 1 pt), DU (HD30, 1 pt) and GC (HC28, 1 pt)	2-DE, LC ESI Q-TOF.	The most overexpressed proteins under oxidative stress overall included: 3 virulence factors (cytotoxin-associated protein A CagA; vacuolating cytotoxin VacA; adherence-associated protein AlpA), 2 antioxidant enzymes (alkylhydroperoxide reductase AhpC and catalase KatA), a serine protease HtrA, an aconitate hydratase, and a fumarate reductase.	(55)
GC (YN8, 1 pt), G (YN14, 1 pt) and DU (YN14, 1 pt)	2-DE and Q-TOF.	Identification of: adenosine triphosphate (ATP)-binding protein; disulfide oxidoreductase B (DsbB)-like protein; N utilization substance A NusA; ATP-dependent protease binding subunit/heat shock protein; hydantoin utilization protein A; seryl-tRNA synthetase, molybdenum ABC transporter ModD, and hypothetical proteins.	(85)
Early GC (1 pt) or chronic G (1 pt)	2D DIGE, MALDI TOF-TOF MS.	Identification of 32 and 14 differentially expressed proteins in HP related to early GC and CG, mostly being antioxidant (superoxide dismutase SodB; catalase Kat A; alkyl hydroperoxide reductases AphC/TsaA; thioredoxin TrxA; nonheme iron-containing ferritin Pfr), memberof the tricarbon acid cycle (isocitrate dehydrogenase Idh; fumarate reductases FrdA, FrdB and FldA; aconitate hydratase 2 AcnB) and heat shock proteins (chaperone and heat shock protein GroEL and heat shock protein ClpB).	(32)
GC and UD (129 strains)*	Protein Chip Arrays, . SELDI TOF MS, 1-DE, LC MS/MS	18 GC biomarkers were selected and 3 of them were purified and identified as: a neutrophil-activating protein NapA, a RNA-binding protein, and a DNA-binding histone-like protein HU.	(86)
G (328)*, DU* (G39)* and GC (10K)*	2-DE, MALDI-TOF MS, Edman degradation.	4 proteins were present only on the 2D map of the strain isolated from DU patients (6-phosphogluconolactonase; S-ribosylhomocysteine lyase; aliphatic amidase; and hypothetical protein HP0697), while 5 were specific for that one isolated from GC patients (hypothetical protein HP0958; transcription elongation factor greA; quinone reactive Ni/Fe hydrogenase large subunit; and NADPH-flavin oxidoreductase RecA protein).	(24)
G (22 pts), GU/DU (24 pts) and GC (25 pts)	2-DE, MALDI-TOF MS, Quadrupole TOF MS.	10 representative proteins were selected, whose expression levels significantly differed among the gastric disease patterns (cytotoxin-associated protein A CagA; urease β subunit UreB; chaperonin GroEL; elongation factor Tu EF-Tu; elongation factor P EF-P; adhesin-thiol peroxidase TagD, and flavodoxin FldA).	(62)
G (80 pts), DU (80 pts) and GC (40 pts)	WB.	OipA positive status was significantly associated with the presence of DU and GC, high HP density, and severe neutrophil infiltration. While, SabA positive status was associated with GC, intestinal metaplasia, and corpus atrophy, and negatively associated with DU and neutrophil infiltration.	(87)
GC (isolates 8, 36, 37 and 46)*	2-DE, MALDI-TOF.	10 proteins showed highly different pI values depending on the HP isolate (cag26 pathogenicity island protein; ATP dependent proteinase binding subunit clpB; 60 kD chaperone groEL; trigger factor tig hemolysin secretion precursor protein hylB; putative transcription regulator; hypothetical protein; superoxide dismutase sodF; NADPH flavin oxidoreductase frxA; peptidoglycan associated lipoprotein precursor PAL omp18; adhesin thiol peroxidase tagD, and non heme ferritin pfr).	(59)
G (4 pts), DU (7 pts), GC (5 pts)	2-DE, N-terminal sequencing.	8 proteins were identified but not specific of any among the analyzed disease (alkyl hydroperoxide reductase TsaA, inorganic pyrophosphatase PpaA, unknown function, two 3-dehydroquinase type II AroD, 3-ketoacid-coenzyme A transferase subunit BScoB, and elongation factor P).	(13)

[a)] asterisk indicates an unspecified number of patients.

Abbreviations: 1-DE, one dimensional electrophoresis; 2-DE, two dimensional electrophoresis; 2D, two dimensional; DIGE, differential in gel electrophoresis; DU, duodenal ulcer; ESI, electro spray ionization; G, gastritis; GU, gastric ulcer; HP, *Helicobacter pylori*; LC, liquid chromatography; MALDI, matrix-assisted laser desorption ionization; nr of pts, number of patients; SELDI, surface enhanced laser desorption ionization; Q-TOF, quadrupole time of flight; TOF, time of flight; WB, western blotting.

Supplementary Table 3. Protein pairs of the Helicobacter pylori strains isolated from patients affected by autoimmune atrophic gastritis (AAG), gastric cancer (GC) or duodenal ulcer (DU), which were labelled with either Cy3 or Cy5 dyes and mixed with the Cy2-labelled internal standard.

Gel nr.[a)]	Patient[b)]	Disease[c)]	Localization[d)]	Cye-Dye [d)]
1	1	AAG	C	Cy3
	4	GC	C	Cy5
2	7	GC	C	Cy3
	4	AAG	C	Cy5
3	1	GC	A	Cy3
	4	AAG	A	Cy5
4	7	DU	C	Cy3
	10	GC	C	Cy5
5	11	GC	C	Cy3
	8	DU	C	Cy5
6	2	DU	C	Cy3
	12	GC	C	Cy5
7	13	GC	C	Cy3
	14	DU	C	Cy5
8	4	GC	C	Cy3
	4	DU	C	Cy5
9	2	DU	A	Cy3
	2	GC	A	Cy5
10	3	GC	A	Cy3
	3	DU	A	Cy5
11	4	DU	A	Cy3
	4	GC	A	Cy5
12	5	GC	A	Cy3
	5	DU	A	Cy5
13	6	DU	A	Cy3
	6	GC	A	Cy5
14	7	GC	A	Cy3
	1	DU	A	Cy5
15	8	GC	A	Cy3
	9	GC	A	Cy5
16	10	GC	A	Cy3
	11	GC	A	Cy5
17	12	GC	A	Cy3
	13	GC	A	Cy5
18	8	DU	C	Cy3
	7	GC	C	Cy5
19	2	AAG	A	Cy3
	3	AAG	C	Cy5

[a)]the number indicates the gel in the Decyder workflow; [b)]the number indicates the AAG, GC or DU patient; [c)]AAG, autoimmune atrophic gastritis; GC, gastric cancer; DU, duodenal ulcer; [d)]A, antrum; C, corpus; [e)]Cye-Dye, cyanine dye.