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COMPARATIVE ANALYSIS OF TWO-DIMENSIONAL ELECTROPHORESIS MAPS (2-DE) OF *Helicobacter pylori* FROM BRAZILIAN PATIENTS WITH CHRONIC GASTRITIS AND DUODENAL ULCER: A PRELIMINARY REPORT

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SUMMARY

Helicobacter pylori is a bacterium recognized as the major cause of peptic ulcer and chronic gastritis. Recently, a proteome-based approach was developed to investigate pathogenic factors related to *H. pylori*. In this preliminary study, *H. pylori* strains were isolated from gastric biopsies of patients with chronic gastritis and duodenal ulcers. A partial proteomic analysis of *H. pylori* strains was performed by bacterial lyses and proteins were separated by two-dimensional gel electrophoresis (2-DE). A comparative analysis was performed to verify a differential protein expression between these two 2-DE maps. These data should be useful to clarify the role of different proteins related to bacterial pathogenesis. This study will be completed using a larger number of samples and protein identification of *H. pylori* by MALDI-TOF mass spectrometry.

KEYWORDS: *Helicobacter pylori*; Proteome; Bidimensional electrophoresis; Chronic gastritis; Peptic ulcers.

Helicobacter pylori is a pathogenic bacterium associated with the etiopathogenesis of universally spread chronic gastritis, peptic ulcers, gastric adenocarcinoma and MALT-lymphoma, mainly in developing countries^{1,5}. Several of its biological aspects have not been revealed yet, in spite of molecular studies about this bacterium. A strategy to improve these studies consists in analyzing the whole protein expressed by the organism in its microenvironment. This is defined as proteome and the methodology is based around the technique of two-dimensional electrophoresis, which is directed towards display of proteins expressed inside gels, followed by protein identification using Peptide Mass Fingerprinting mass spectrometry (MALDI-TOF-MS)^{7,8,9}.

Recently, some reports have described a comparison among 2-DE proteome maps of clinical isolates obtained from patients who had peptic ulcers and chronic gastritis. It has been found that the main proteins have a different expression in these 2-DE maps, including GroEL (a protein that increases urease enzyme activity) and SodF (an enzyme involved in free radical and hydrogen peroxide catabolism)^{2,3}. In this work, we carried out a comparative analysis of 2-DE maps of *H. pylori* from Brazilian patients, in order to find molecular targets related to its pathogenic mechanism in clinical isolates derived from duodenal ulcers and gastritis.

H. pylori strains were isolated from gastric mucosa biopsies of two patients who had not been previously treated, submitted to upper

gastrointestinal endoscopy at Gastrocentro, UNICAMP. Bacterial isolation and protein extraction were performed according to our previous reports^{6,8}.

The samples were submitted to isoelectrofocusing (IEF) solubilized with 8M urea, 4% CHAPS, 70 mM DTT, ampholine linear pH gradient 4-7 (pH 4-7L) 1.5% and 0.001% bromophenol blue. At this stage, 64 µg of each protein sample were applied to polyacrylamide dried strips, pH gradient 4-7 L (immobilized pH gradient - IPG). The first dimension of 2-DE was performed on an Amersham Biosciences Electrophoresis System accumulating 96 kVh in the electrophoresis condition. Following isoelectric focusing, material in the IPG strip was exposed for 8 min to an equilibration solution containing SDS/DTT and for 12 min to another solution containing SDS/Iodoacetamide. The strips were covered with 0.5% agarose heated at 70 °C and submitted to the second dimension of 2-DE using SE-600 electrophoresis apparatus and 12.5% polyacrylamide gels. After staining with silver nitrate, the gels were scanned and analyzed by Image Master 2D v3.1 Elite software (GE - Amersham Biosciences)^{8,9}.

The proteins were characterized by different levels of expression and lack of appearance of other spots. Our results showed that four spots of the Gastritis 2-DE gel were not seen in ulcer one (Fig. 1 - spots 4, 7, 8 and 11 of sample 2); four spots were visualized in Ulcer 2-DE gel but they were not seen in Gastritis one (Fig. 1 - spots 3, 15,

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Table 1
Corresponding spots of ulcer and gastritis samples, their relative volume and ratio

ULCER (SAMPLE 1)		GASTRITIS (SAMPLE 2)		ULCER/GASTRITIS Volume ratio
SPOT	Relative volume	SPOT	Relative volume	
1	25.042	5	65.227	0.38
2	31.925	6	14.990	2.13
3	27.489	-	-	-
4	362.246	1	278.056	1.30
6	138.562	2	70.223	1.98
15	120.032	-	-	-
19	138.474	-	-	-
20	443.417	9	461.052	0.96
21	93.903	10	251.221	0.38
22	69.523	-	-	-
-	-	4	70.620	-
-	-	7	37.123	-
-	-	8	105.962	-
-	-	11	9.603	-

Description of spots on ulcer (sample 1) and gastritis (sample 2) gels and their respective volumes. The corresponding spots occupy the same position (they have the same pI and MW) in the gels. The relative volumes represent the indirect protein expression measured by the Software Image Master 2D.

19 and 22 of sample 1). Finally, six spots had different expression, measured by their relative volume unit (Fig. 1 and Table 1).

A proteome map and the identification of some *H. pylori* proteins (pH range 3-10 and 4-7), previously built by our group (data not shown), supplied a standard protein expression from a patient without any gastric

disease. It was useful to compare the protein expression of *H. pylori* isolated from gastric mucosa of patients with gastric diseases and healthy subjects. The identification of the GroEL protein with pI 5.55, MW 58264.4, Gene/ORF-TIGR HP0010 and access number P42383 (ExpASY - Swiss-Prot and TrEMBL® - <http://us.expasy.org/sprot>), at the reference proteome map 3-10, is preliminary information that indicates this protein could become a target for subsequent research. These results are similar to data described by KRAH *et al.*⁴ who reported that the pattern expression of GroEL was different in gastrointestinal diseases.

The distinct patterns of protein expression demonstrated in each sample suggest that *H. pylori* bacteria are able to express proteins distinctly. However, to confirm whether those gastrointestinal diseases are related to different patterns of *H. pylori* protein expression, further studies using a pool of bacterial proteins should be carried out. The use of MALDI-TOF-MS technology will provide identification of bacterial proteins. This approach can be useful to clarify the pathogenic mechanisms of *H. pylori* related to gastrointestinal disorders.

RESUMO

Análise comparativa de mapas de eletroforese bidimensional (2-DE) de *Helicobacter pylori* de pacientes brasileiros com úlcera duodenal e gastrite crônica: relato preliminar

O *Helicobacter pylori* é uma bactéria reconhecida como a principal causa de úlcera péptica e gastrite crônica. Recentemente, o proteoma do *H. pylori* tem sido desenvolvido visando identificar fatores patogênicos relacionados ao microorganismo. Neste estudo preliminar, cepas de *H. pylori* foram isoladas de fragmento de mucosa gástrica de pacientes com úlcera duodenal e gastrite crônica. Posteriormente, realizou-se uma análise proteômica parcial dessas cepas, através da

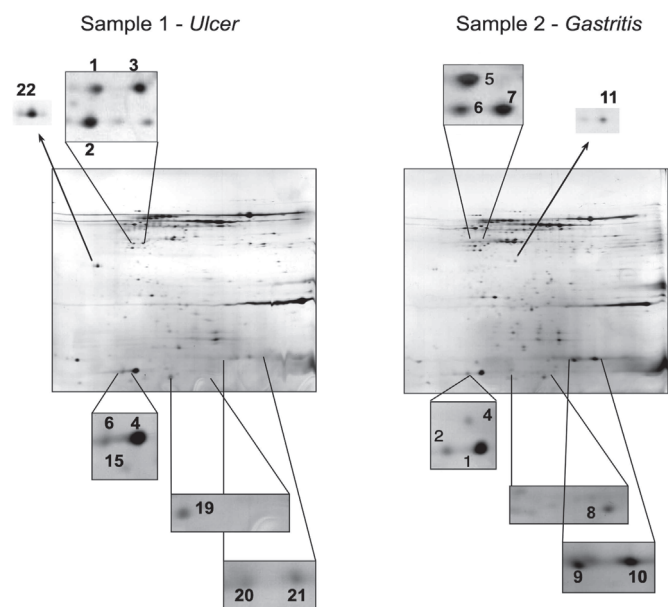


Fig. 1 - Proteomic Maps comparing the 2-DE gels from a patient with duodenal ulcer (Sample 1) and another with gastritis (Sample 2). The numbers in the spots are explained in Table 1. The highlighted regions correspond to areas containing differential expression of proteins.

lise bacteriana e da separação de proteínas através da eletroforese de duas dimensões (2-DE). Por análise comparativa, foi possível verificar a expressão protéica diferencial entre os dois mapas 2-DE obtidos. Os dados poderão ser úteis para esclarecer a importância de diferentes proteínas relacionadas à patogênese da bactéria. Este estudo será complementado utilizando um maior número de amostras e a identificação protéica do *H. pylori* através da espectrometria de massa do tipo MALDI-TOF.

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