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Comparative Proteome Analysis of *Helicobacter pylori* Strains Associated with Iron-Deficiency Anemia

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Helicobacter pylori is a gram-negative, microaerophilic bacterial pathogen that colonizes the mucosal layer overlying the gastric epithelium of the human stomach, and *H. pylori* infection has been associated with various severe gastric diseases, including chronic gastritis, peptic ulcers and gastric cancer. In addition, *H. pylori* infection was recently linked to iron-deficiency anemia (IDA), particularly in adolescents and premenopausal women [1], while Choe *et al.* showed that IDA in *H. pylori*-infected patients was refractory to iron therapy and only reversed after *H. pylori* eradication even without iron supplementation [2]. Although several hypotheses have been proposed for the pathogenesis of *H. pylori*-related IDA, the exact mechanism of the pathogenesis of *H. pylori* infection-associated IDA has not been clearly elucidated.

Pathogenic microorganisms can be compared both at the genomic and proteomic levels, but differential expression at the proteomic level is thought to be more relevant to the difference of phenotypes. Accordingly, comparative proteomics of bacterial pathogens has been widely applied to obtain information on strain variability, environmental influences and gene functions.

In the present study, using proteomic cluster analysis, we determined whether infecting *H. pylori* strains are correlated with the prevalence of IDA. A total of 15 strains, 8 non-IDA and 7 IDA strains, were isolated from antral biopsy specimens of Korean adolescents, cultured under iron-rich and iron depleted conditions and analyzed for their protein expression profiles by 2-dimensional electrophoresis (2-DE). A master gel was run with mixtures of equal aliquots from 16 strains including *H. pylori* 26695. The resulting protein expression profiles displaying a total of 1746 spots were used as a protein expression reference map and each spot on the map was numbered. Protein spots on 2-DE gels of each strain were matched against the reference map and matched spots were assigned numbers. Although the comparison of the protein expression profiles of the strains displayed main spots at the same position, several major changes in position and spot intensity were observed, indicating a high heterogeneity among the *H. pylori* strains. The number of spots matched to the reference map varied among *H. pylori* strains, ranging from 13% to 19.8% of the total spots in the reference map.

To determine whether the protein expression profiles of *H. pylori* strains distinguish between non-IDA and IDA strains, pairwise comparisons among the *H. pylori* strains were made. Since the capability of *H. pylori* to compete with a host for environmental iron is presumed to contribute to the pathogenesis of *H. pylori*-associated IDA, 129 spots whose expressions appeared to be modulated by iron were randomly selected for the analysis. In order to build the similarity matrix for *H. pylori* strains, distances between the protein expression profiles of two strains were determined and a neighbor joining tree was constructed as implanted in the Mega software package [3]. The phylogenetic tree obtained revealed that the IDA strains formed a separate cluster from the non-IDA strains with two non-IDA strains between the clusters (Fig. 1A). The *H. pylori* strain 26695 was observed to belong to the non-IDA cluster. This result indicated that the expression profiles of iron-regulated proteins may differentiate the two groups. Since A19, an IDA strain, was located in the non-IDA cluster, however, factors other than iron-responsiveness was thought to play a part in determining the IDA phenotype. Therefore, spots showing differential expression between any two strains were further selected, and a total of 189 spots including the 129 spots were then used for a phylogenetic analysis as above. The phylogram presented in Fig. 1B showed that the non-IDA and IDA strains were separated into two clusters. Two non-IDA strains, G21 and G22, were present between the clusters as in the tree constructed on the basis of the 129 spots. These results indicated that the IDA and non-IDA strains could be distinguished by their protein expression patterns, suggesting that the strains within each group are more genetically related to each other than to strains in the other group.

The 189 spots were clustered using the 'Cluster' software [4] according to similarity in expression pattern between the non-IDA and IDA groups and those predominantly expressed in the IDA strains were identified by MALDI-TOF analysis. Total 18 protein spots identified comprised diverse groups of proteins with functions including chemotaxis, cell division, energy metabolism, fatty acid metabolism, transcription, translation, and biosynthesis of heme, amino acid, polysaccharide and lipopolysaccharide. Among these, *amiE* and *envA* have been shown to be repressed by iron in our previous study and *hyuA* activated by Fur [5].

In summary, these data indicate that the non-IDA and IDA strains can be distinguished by their protein expression profiles, suggesting the relevance of infecting *H. pylori* strains to their clinical phenotypes. The data also suggest that protein spots expressed at significantly higher abundance in IDA strains might be involved in the development of IDA in *H. pylori*-infected patients and should provide useful information for further studies on the pathogenesis of *H. pylori*-associated IDA.

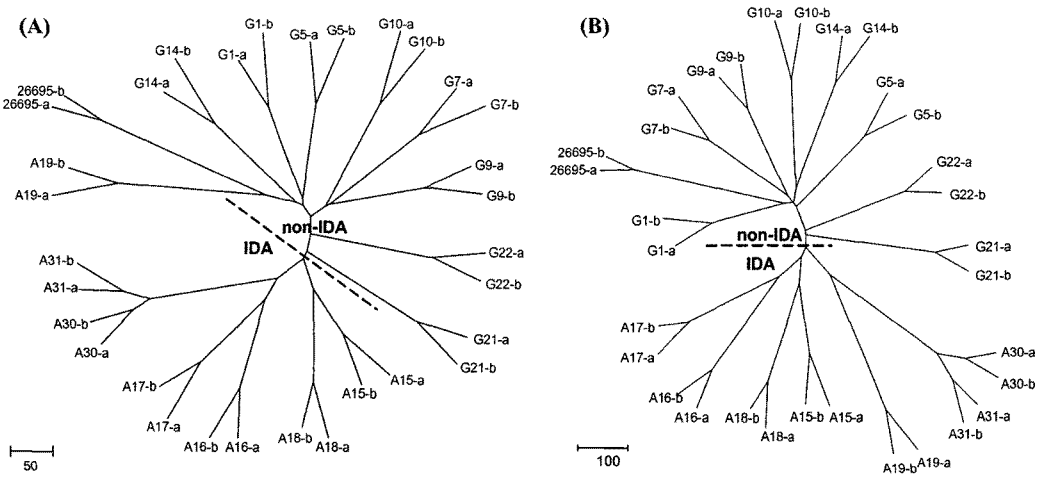

Figure 1. Phylogram of *H. pylori* strains based on the overall protein expression profiles. The overall distances between *H pylori* strains were determined based on the intensities of 129 protein spots (A) and 189 spots (B). A designates strains isolated from IDA patients; G, strains from gastritis patients without IDA. a, bacteria grown on BBS10 plates; b, bacteria grown on BBS10 containing desferal.

References

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