Novel protein antigen (JHP940) from the genomic plasticity region of *Helicobacter pylori* induces TNF-alpha and Interleukin- 8 secretion by human macrophages

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Background: The gastric pathogen *H. pylori* is one of the most successful pestilences of the mankind, which infects almost half of the world population. However, a small fraction of infected individuals experience *H. pylori* associated diseases such as gastritis, peptic ulcers and more rarely, the gastric adenocarcinomas (1). Just like any other microbial genome, *H. pylori* genome also contains a wealth of genes with no known function; many of them unexplored as yet. Some loci within the plasticity region have been thought previously to serve as markers of virulence (gastritis or cancer) and hence the assumption that these may be strain specific genes (2,3), which might be gained or lost or rearranged at liberty during adaptation to a new host. However, these putative markers have not been functionally characterized as yet.

Methods:

Selection of Orf:
The locus jhp940 was primarily selected based on dot blot hybridization study of Occhialini. Amino acid sequence was searched for possible immunogenic epitopes using expert software (DNAstar-Lasergene, NCBI-blast, Protean etc).
Genomic PCR amplification was carried out using 20 *H. pylori* DNA (irrespective for disease status) representing each geographic region to analyze the distribution of jhp940. Stability of locus jhp940 was checked in serial isolates obtained a decade apart from different niches (corpus and antrum) of a single patient.
Gene was cloned in pRSETA expression system (Invitrogen, Carisbad, CA) and the protein was purified using Ni-NTA column (Qiagen, Hilden, Germany). Homogeneity of the protein was confirmed on 10%SDS-PAGE. Endotoxin contamination was removed using PolymixinB (Sigma, USA).

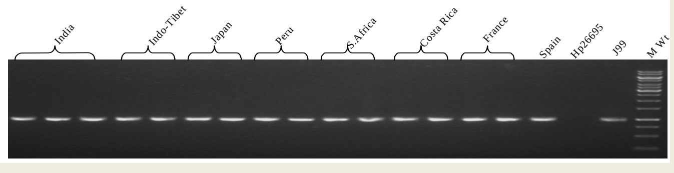
T-cell response: Thp1 cells (ATCC, USA) grown in RPMI 1640 (Invitrogen) supplemented with10% fetal bovine serum (FBS)(v/v) and 1% antibacterial and antimycotic solution were differentiated into adherent macrophage-like cells using 5ng/ml phorbol 12-myristate 13- acetate (PMA; Sigma, USA).
Peripheral blood mononuclear cells were isolated from the heparinized venous blood drawn from the voluntary donor (s) using Ficoll-histopaque density gradient centrifugation. Cell viability was checked by Trypan blue dye exclusion method and was found to be 90%. Differentiated macrophages and PBMCs were stimulated with varying concentration of protein 0.1µg, 0.25µg, 0.5µg, 0.75µg, and 1.0µg/ml. Unstimulated and proteinase K treated cells (LPS; Sigma), were used as negative controls whereas LPS treated cells (E.coli, Sigma, USA) was used as positive control. Culture supernatants were collected at 24 hours and stored at -80^{o}C until assayed.

Cytokine estimation:
Induction of cytokines (IL-8 and TNF alpha) was estimated using commercially available OptEIA Elisa kit (BD Biosciences, San Jose, CA). The plates were read in ELISA reader at 490nm.
Students-*t* test was used to analyze the data and values were represented as standard error of mean (SEM), *P* values less than 0.05 were considered as statistically significant

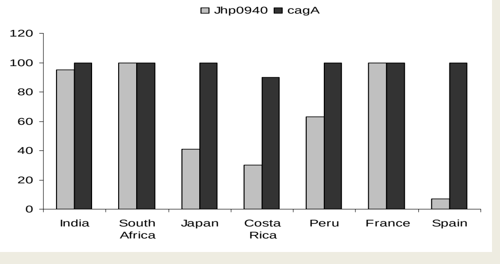
Results:

JHP940 is a putative virulence factor which is broadly conserved and present in significant proportion of strains representing different geographic regions (fig-1). Also, the gene is highly conserved in the strains isolated from different niches across a period of ten years. Its prevalence is independent of cagA and disease status (fig-2).
As TNF-alpha and IL-8 are the most potent stimulator of *H. pylori* associated pathogenesis we evaluated the potential of our target to stimulate the secretion of cytokines. JHP940 elicits a strong cytokine response in Thp-1 differentiated macrophages and PBMC in a dose dependent manner (fig-4). Our comparative analysis with LPS portrays Jhp940 as a more potent stimulator of cytokines.

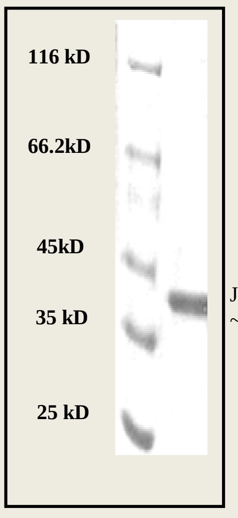
Geographic distribution of Locus jhp940



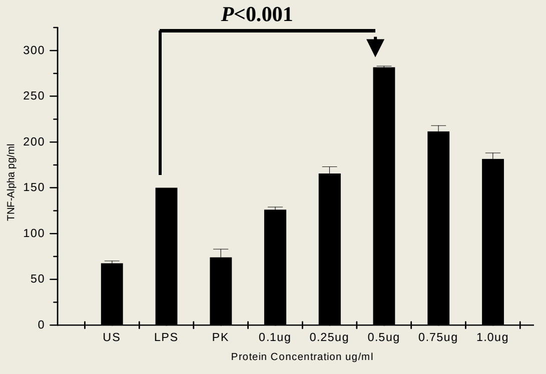
% Geographic prevalence ofjhp940 and cagA

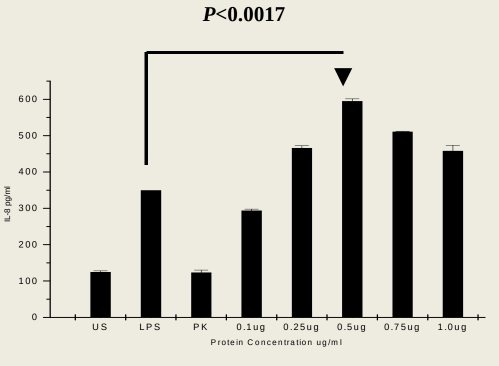


Recombinant JHP940 purified on Ni-NTA column

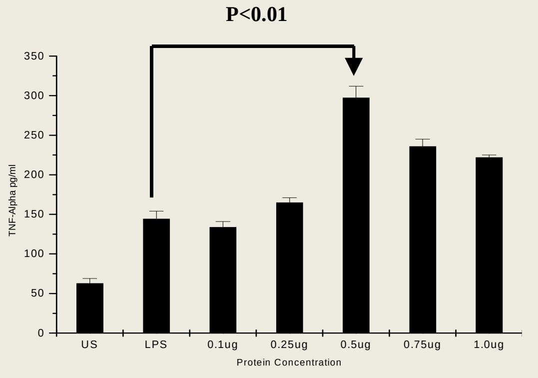


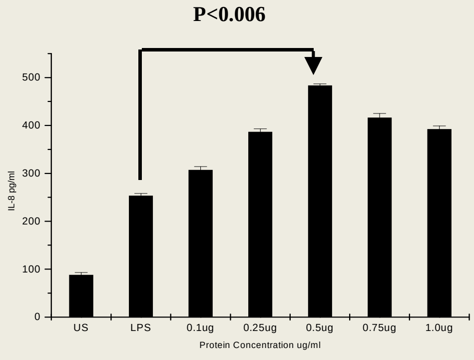
JHP940
~36kDa





Secretion of proinflammatory cytokines estimated in culture supernatant following stimulation of Thp-1 differentiated macrophages with recombinant protein.





Estimation of TNF-alpha and IL-8 secreted in the culture supernatant following stimulation of human PBMC with recombinant JHP940

Conclusion:

Identification of JHP940 in a biologically active form as a potent proinflammatory cytokine stimulator has consolidated the assumption that a pool of different virulence factors other than the classical one plays significant role in *H. pylori* mediated pathogenesis.

References:

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2) **Occhialini, A., A. Marais, R. Alm, F. Garcia, R. Sierra, and F. Mégraud. 2000.** Infect. Immun. **68:**6240-6249.

3) **Santos, A., M. Queiroz, D. M. Ménard, A. Marais, A. Rocha, G. A. Oliveira, C. A. A. M. M. Ferreira Nogueira, M. Uzeda, and F.Mégraud.** 2003. J. Clin. Microbiol. **41:**1651-1652

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