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Expression of Urease B from Iranian Helicobacter pylori in Apoplast of Tobacco - Neda Maleki Tabrizi- University of Tehran

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1. Background

Helicobacter pylori is a Gram-negative bacterium, which was first isolated by Marshall and Warren from the gastric mucosa of patients with gastritis and peptic ulcers. Helicobacter pylori is the main cause of chronic infection in gastric mucosa, affecting 50% of the world's population. Recently, H. pylori has been shown to cause other complications, such as gastric ulcers, duodenal inflammation, and gastritis. According to the literature, a considerable proportion of patients, infected with H. pylori, may not develop gastroduodenal diseases and could remain asymptomatic for a long period. On the other hand, long-term infections raise the risk of conditions such as adenocarcinoma and gastric lymphoma.

Several studies have confirmed H. pylori as an ultimate carcinogenic agent. The mortality rate, associated with Helicobacter pylori infection, has been estimated at 1 case per 34 infected men and 1 case per 60 infected women. Helicobacter pylori produces a large amount of urease (10% - 15% of the whole protein weight) which is required for the survival and pathogenesis of H. pylori. Many enzymes reduce the acidity of the immediate environment of H. pylori by producing ammonia and carbonate from urea. Helicobacter pylori urease is a multimeric enzyme with a molecular weight of 550 kDa, which is collected from two separate subunits of UreA (29.5 kDa) and UreB (66 kDa). Recently, researchers have been concerned with the development of vaccines against H. pylori infections. Among various candidate antigens, UreB is considered to be the most effective, as immunization of mice with purified UreB results in higher immunogenicity and protection, unlike Urea. Selection of urease as an immunization target is based on the fact that membrane proteins often elicit an immune response.

In this study, we used a prediction software program with high performance for epitope mapping. With several specific epitopes of B lymphocytes, located next to each other, this program selected the amino acid fragment of UreB gene. Moreover, the antigenic fragment was expressed and purified as a recombinant protein of Escherichia coli BL21(DE3) strain. This study aimed to present a fragment of recombinant UreB (rUreB) with significant antigenic properties, using relevant software programs.

2. Objectives

The purpose of this study was to provide a recombinant vector, comprised of the antigenic region of UreB from H. pylori, using the immunodominant epitopes of the antigen instead of the total sequence and expression in E. coli. Also, we aimed to determine its antigenicity as a vaccine candidate and assess its efficacy in the serological diagnosis of H. pylori infection in humans.

3. Methods

3.1. Preparation of Bacteria, Plasmids, and Other Reagents

In this study, H. pylori was cultured from the biopsy of dyspeptic patients, undergoing routine diagnostic endoscopy. Prior to biopsy, informed consents were obtained from all the patients for participation in the study. Culture conditions were determined, based on the findings of previous reports. The isolated biopsy specimens were cultured on Brucella agar (Merck, Germany), containing trimethoprim (5 µg/mL), vancomycin (10 µg/mL), and amphotericin B (2.5 µg/mL), supplemented with 5% sheep blood.

After incubation under microaerophilic conditions for 3 - 5 days at 37°C (CO_2: 10%), the grown bacteria were identified as H. pylori via routine microbiological tests, including Gram staining, as well as oxidase, urease, and catalase tests. For further evaluation, we used pET-32a and pBSK vectors (Novagen Inc., USA). Moreover, the pET-32a vector was used to produce fusion proteins with a 6-histidine tag and an N-terminal T7 epitope tag.

These additional amino acids increased the weight of the produced protein by up to 20 kDa. In this study, restriction enzymes and DNA ligase were purchased from Fermentas (Lithuania), and E. coli DH5α strain (Stratagene, USA) was used for the initial cloning. In addition, recombinant pET-32a (pET-32a UreB) was transformed into E. coli BL21(DE3)pLysS (Novagen, USA) as the host strain of bacteria. For routine bacterial culture, we used the Lysogeny broth (Luria-Bertani broth, LB; Sigma, USA). The required antibiotics, including ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL; Sigma, USA), were added to the LB medium (10). The chemicals were purchased from Merck (Germany), and the enzymes were obtained from Fermentas (Lithuania) and SinaClon Co.

Results: The sequencing results of PCR were indicative of UreB gene cloning into the recombinant plasmid. Production of the protein was induced by isopropyl β-D thiogalactopyranoside (IPTG), and the expressed protein was purified via dialysis, using the Ni-NTA kit. In addition, the recombinant protein with a molecular weight of 42 kDa was recognized by antibodies in Western blotting.

Conclusion: The evaluation of antibodies was indicative of high antigenic properties for the immunogenic fragment, predicted by immunological bioinformatics. Therefore, UreB recombinant protein might be a proper antigen for the development of vaccines against H. pylori and other diagnostic kits.