Lactococcus lactis as an oral vector for cloning of heat shock protein A from Helicobacter pylori

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Key words: Helicobacter pylori, Heat shock protein A, Probiotic, Lactococcus lactis.

http://dx.doi.org/10.12692/ijb/6.3.410-415 Article published on February 16, 2015

Abstract

Lactococcus lactis, a safe probiotic bacterium would be one of the potential candidates for cloning. The aim of present study was to clone H. pylori heat shock protein A (hspA) in Lactococcus lactis for application in immunological studies. hspA gene sequence of H. pylori 26695 containing His-tag-Opt was constructed and was inserted into E. coli-L. lactis shuttle vector pNZ8148, transformed into E. coli MC1061, and its insertion was confirmed by PCR. The purified recombinant plasmid was introduced into L. lactis NZ9000 by electro-transformation. The presence of hspA gene in pNZ8148 shuttle vector, as well as in L. lactis NZ9000 transformants was confirmed by enzymatic digestion, PCR and sequencing of cloning products. Due to the safety and importance of probiotic bacteria, the recombinant L. lactis NZ9000 expressing H. pylori hspA protein would be a promising oral vaccine candidate against H. pylori.

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**Introduction**

*Helicobacter pylori* colonizes human gastric mucosa in about half of the world's population and results a variety of diseases including chronic gastritis, gastric and duodenal ulcer, gastric carcinoma as well as mucosa-associated lymphoid Tissue (MALT) lymphoma (Fischbach et al., 2004).

The most accurate way to resolve definitively the problems associated with *H. pylori* infection would its prevention via vaccination of individuals, especially in the regions with high prevalence of infection.

Lactic acid bacteria or probiotics exist in various niches, including gastrointestinal tract (Pfeiler and Klaenhammer, 2007). Previous studies have shown the beneficial effects of probiotic bacteria on human health and its potential role as probiotic in patients with *H. pylori* infections (Franceschi et al., 2007; Lionetti et al., 2010; Thomas and Versalovic, 2010).

So, development of the recombinant probiotics expressing proteins of *H. pylori* may be useful as preventive vaccine against *H. pylori* infections.

There are a few report concerning efforts on development of recombinant probiotic bacteria expressing the adhesin Hpo410 of *H. pylori* (Hongying et al., 2014; Zhu et al., 2010) and recombinant *Lactococcus lactis* expressing urease subunit B as an oral vaccine against *H. pylori* in mice model (Gu et al., 2009).

Heat shock protein A (hspA) is induced in response to unfavorable environmental conditions such as heat shock, oxidative stress and exposure to heavy metals. This protein exists in all domains of life and acts as a molecular chaperone (Horwich et al., 2007). hspA has common characteristics such as high degree of homology, strictly conserved protective sequence with molecular weight of 10-30 kDa. *H. pylori* hspA protein is composed of 118 amino acids with two domains A [N-terminal domain (1-91)] and B [C-terminal domain (92-118)]. It was demonstrated that urease activity increases fourfold when co-expressed with hspA in *E. coli*, probably via its domain B which acts with the nickel-binding property (Kansau et al., 1996). Although urease would be one of the most important virulence factors of *H. pylori*, hspA is an immunogen protein and the main target of bismuth-containing anti-cancer drugs against *H. pylori* (Bumann et al., 2004; Eamranond et al., 2004).

The purpose of this study was to use *L. lactis* NZ9000, a probiotic bacterium as a vehicle for delivering hspA protein of *H. pylori*, for future immunological purposes.

**Material and methods**

**Bacterial strains and plasmids**

Bacterial strains were *Escherichia coli* Top10, *E. coli* MC1061 and *Lactococcus lactis* NZ9000 (MoBiTec, Germany). The plasmids were pUC57 (Gene Scipt, USA) and pNZ8148 (MoBiTec, Germany).

*E.coli* Top10 and *E. coli* MC1061 strains, selected for transformation, were grown in Luria-Bertani broth (LB, Merk, Germany) at 37 °C; *Lactococcus lactis* NZ9000 was grown in M17 medium (Merk), supplemented with 0.5% glucose at 30°C supplemented (when necessary) with antibiotics at the concentrations of 50 μg/ml, 40 μg/ml, and 10 μg/ml for ampicillin, chloramphenicol and erythromycin (Sigma, USA), respectively.

**Construction and identification of a shuttle vector containing His-tag and optimized (Opt) hspA**

On the basis of hspA sequence from *H. pylori* ATCC 26695, the 6- His-tag-Opt-hspA with endonuclease sites for *HindIII* and *NcoI* were synthesized by GenScript Company, USA. At the first time, the 6- His-tag-Opt- hspA construct was cloned into pUC57 which produced a shuttle vector.

PUC57- Opt-hspA and pNZ8148 as cloning vector were digested with *HindIII* and *NcoI* endonucleases in optimized conditions and were ligated (Sambrook et al., 1989). The recombinant pNZ8148 transformed into the competent *E. coli* MC1061 on the basis of Promega protocols and application Guide, (3rd edition, p.45-46) by Tris- calcium chloride and
Rubidium chloride methods. The recombinant colonies were isolated on LB agar containing 40 µg/mL of chloramphenicol and incubation at 37 °C. Recombinant plasmids from transformant colonies were extracted by DNA extraction kit (Dena zist, Mashhad, Iran) and were identified by digestion with NcoI and HindIII as well as colony PCR using the designed primers F(5'-3'): CCATGGGGAGCTCAATAC; R(5'-3'): CTCTAGACAGCAGTTCC at the optimised conditions as follow: preheating at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, the final extension was performed at 72 °C for 10 min. The result of PCR was subjected to DNA sequencing.

Producing of recombinant L. lactis NZ9000 by electro-transformation

In order to obtain a L. lactis NZ9000 expressing H. pylori hspA gene, the recombinant plasmid of pNZ8148-hspA was transformed electrically into the competent L. lactis NZ9000 strain. The electroporation conditions were 2000 V, 25 µF, 200 Ω (Bio-Rad Genepulsercat, number 165-2100). The recombinant colonies were isolated on GM17 plates containing 40 µg/mL of chloramphenicol, following incubation at 30 °C. The recombinant colonies were identified by colony PCR as noted above.

Results

Double Digestion

The recombinant pUC57 plasmid digested by two restriction enzymes HindIII and NcoI and the results produced two fragments as shown in figure 1.

Sequencing

Sequencing of Opt-hspA in pNZ8148 shuttle vector showed that it corresponded correctly to the inserted sequences which were comparable with that of hspA from H. pylori ATCC 26695.

Electroporation

OPT-hspA-pNZ8148 was transformed into E. coli MC1061 in the experimental conditions optimized by us. The result of electrophoresis of double digested recombinant plasmid recovered after electro-transfert with HindIII and NcoI showed the presence of hspA gene (Figure 2).

Fig. 1. Double digestion of recombinant pUC57 by HindIII and NcoI. Lane 1: No digested recombinant pUC57, Lane 2: double digest of recombinant pUC57, Lane 3: DNA Marker III.

Fig. 2. Double digestion of recombinant pNZ8148 with HindIII and NcoI. Lane 1: pNZ8148 and OPT-hspA. Lane 2: Single digestion of PNZ8148, Lane 3: DNA Marker.

Colony-PCR

After producing the recombinant L. lactis NZ9000, the recombinant colonies were confirmed by direct colony PCR and presence of product with 438 bp, which was comparable with the primary synthesized hspA was detected (Figure 3).

Fig. 3. Colony PCR of recombinant L. lactis. 6: ladder 100 bp (Fermentas). Lane 2: negative control, other lanes were recombinant colonies (438 bp).
Discussion
The current treatment of H. pylori infections includes a combination therapy containing two or three antibiotics plus a proton pump inhibitor (Sugimoto et al., 2014). Emergence of antibiotic-resistant or multidrug-resistant H. pylori especially in the developing regions, requires development of a safe vaccine for prevention of H. pylori infections (Bai et al., 2004; Every et al., 2011; Ferrero et al., 1995; Garhart et al., 2002; Sugimoto et al., 2014). Multiple investigations have been focused on the trialing various H. pylori antigens in animal models (Bai et al., 2004; Every et al., 2011; Ferrero et al., 1995; Garhart et al., 2002; Sugimoto et al., 2014) however, no commercial vaccines have been introduced against H. pylori infections.

Probiotics are living microorganisms that provide a health benefit to host by different mechanisms including activation of the gut mucosal immunity (Isolauri et al., 2001), production of substances that prevent infection or substances with food values (Vitamins or minerals) and interaction with pathogenic bacteria (Reid and Hammond, 2005; Sanders et al., 2010). Development of a shuttle vector able to be expressed in L. lactis would be promising for cloning process since the problems encountered in traditional cloning system such as presence of inclusion bodies is omitted. Furthermore, as a Gram positive bacteria L. lactis do not produce endotoxins. In addition, its genome is about half of the size of the E. coli genome so it may have a delivery vehicle with fewer contaminating proteins (Fischbach et al., 2004).

In present work, we experimented a well-defined shuttle vector for expression of the hspA protein of H. pylori in L. lactis (Mierau and Kleerebezem, 2005). Cloning of H. pylori hspA gene and its expression in L. lactis is important since it may be one of the ideal vaccine candidates for H. pylori infection. This importance may be related to its safety as well as its conserved character among clinical isolates (Mierau and Kleerebezem, 2005). Although, previous studies on hspA have shown that it is an essential protein with good immunogenicity, more studies on the mechanisms of immunological response to this protein is required to clarify its precise role in H. pylori pathogenesis (Suerbaum et al., 1994; Bumann et al., 2004). So, application of a L. lactis as an oral vector may help to clarify better this role.

L. lactis as probiotic bacteria, able to express H. pylori hspA may also be used as a good supplement in prevention of H. pylori infections by its beneficial effects. It can also play a role in activating the effective immunity responses. However, more clinical studies are required for evaluation of the hspA expression and its experimentation in animal model. This first work and its results may be an experimental foundation for development of the food-grade vaccine against H. pylori infection.

Acknowledgement
Alzahra University, Tehran, Iran, have supported this work.

Conflict of interest
The authors have no substantial financial or commercial conflicts of interest with the current work or their publication.

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