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A Novel Lactic Acid Bacteria Display System and its Application

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A *Bacillus subtilis chungkookjang* secreting high viscous poly-γ-glutamic acid (γ-PGA) was isolated from the traditional Korean fermented bean-paste, Chung-Kook-Jang. Poly-γ-glutamic acid (γ-PGA) is a polypeptide that glutamate is polymerized via the γ-amide linkage and is synthesized by the poly-γ -glutamate (γ-PGA) synthetase complex consisting of pgsB, pgsC, and pgsA (pgsBCA system). The pgsBCA system associated with bacterial cell membranes, synthesizes an extremely large polymer with the molecular mass of over 10,000 kDa and is predicted to integrate into the outer membrane of the *Bacillus subtilis* surface. Thus we designed a new surface display system by introducing cell surface protein pgsBCA into the *E.coli-Lactobacillus* shuttle vector (pHCE1LB), which include the high constitutive expression promoter to facilitate high expression of proteins without IPTG induction. We checked the possibility that cell surface protein, pgsBCA is used as an anchor protein by investigating the expression of the S epitope of hepatitis B virus protein fusion to pgsBCA motif. Cell fractionation and fluorescence-activated cell sorting analysis were performed to monitor HBs epitope expression on the surface of *Lactobacillus casei*.

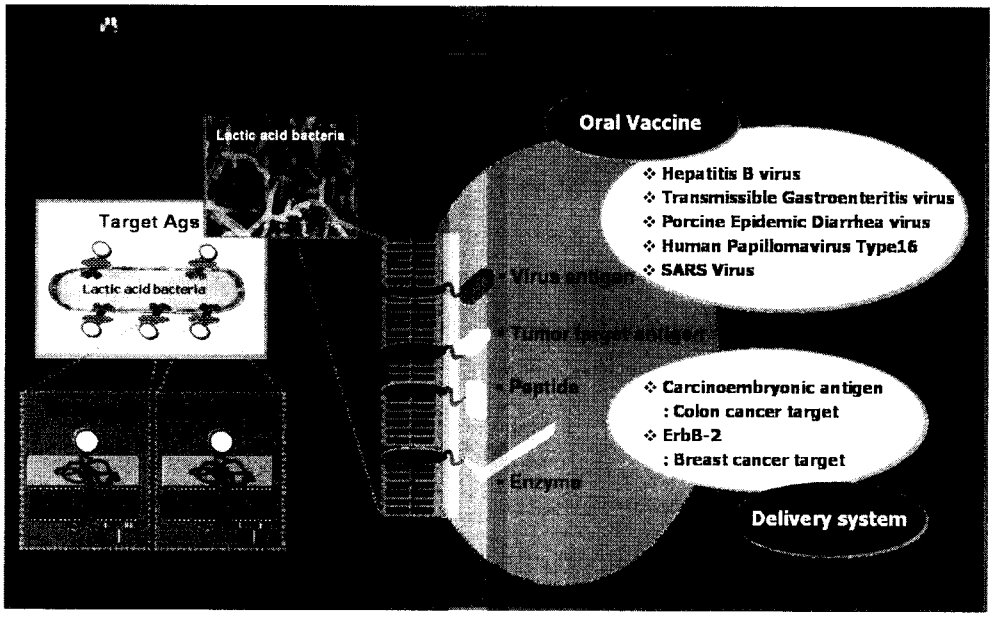
Lactic acid bacteria in general and strains of *Lactobacillus* particularly have a variety of properties which make them attractive candidates for oral vaccination purposes, such as GRAS (generally recognized as safe) statue, adjuvant properties, cytokine secretion, induction of mucosal and systemic immune response and low intrinsic immunogenicity. So, the immunogenicity of surface-displayed eHBs on the recombinant live *Lactobacilli* was assessed in mice inoculated intranasally and orally. Inoculation resulted in significantly level of eHBs-specific serum IgG and intestinal, bronchoalveolar IgA antibody. Thus, this new *Lactobacillus* surface display system may provide an effective mean for inducing mucosal and systemic immune response.

For the diverse application of *Lactobacillus* display system, we developed *Lactobacilli* expressing the antigens of porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) which is the agent of swine infecious diarrhea disease. First, Western blot and fluorescence-activated cell sorting(FACS) analysis were performed to monitor TGE and PED viral antigens expression on the surface of *Lactobacilli*. The immunogenicity study of surface-displayed TGE and PED viral antigens on the live *Lactobacilli* was performed in orally immunized mice and swine. Inoculation resulted in significantly increased level of serum IgG and mucosal IgA antibody specific to TGE and PED viral antigens.

Severe Acute Respiratory Syndrome (SARS) is a newly emerging human disease. A novel coronavirus has been identified as the etiological agent of SARS and designated as SARS coronavirus (SARS-CoV). Becuase SARS is coronavirus infected by similar route with TGEV and PEDV, we developed surface-displayed SARS viral antigens (nucleocapsid and spike) on the live *Lactobacilli* and tested its immunogenicity assessed in mice inoculated orally and intranasally.

Also, for the development of prophylatic and therapeutic vaccine of human papillomavirus type 16 (HPV-16) which is the predominant etiologic agent of cervical cancer, we checked the immunogenicity of surface-displayed HPV-16 viral antigens (capsid L1 and E7) on the live *Lactobacilli* in mice inoculated orally.

The results indicate that the strategy developed in this study may be promising to generate effective live oral vaccines to induce protective mucosal and systemic immune responses against infectious agents in the future.



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