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Construction of Phix174 gene E mediated lysis system for generation of *Salmonella Enteritidis* ghost, and its evaluation as a vaccine candidate for the immunogenicity and protective efficacy against avian salmonellosis

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A new strategy to develop an effective vaccine is essential to control food-borne *Salmonella enterica* serovar Enteritidis infections. Bacterial ghosts (BGs), which are non-living, Gram-negative bacterial cell envelopes, are generated by expulsion of the cytoplasmic contents from bacterial cells through controlled expression using the modified cI857/λ PR/gene E expression system. During our initial studies, we have generated S. Enteritidis ghost using the antibiotic resistance gene containing pJHL99 lysis plasmid carrying the mutated lambda PR37-cI857 repressor and PhiX174 lysis gene E. Temperature induction of the lysis gene cassette at 42°C revealed quantitative killing of S. Enteritidis. In the development of genetically inactivated bacterial vaccines, plasmid retention often requires the antibiotic resistance gene markers, the presence of which can cause the potential biosafety hazards such as the horizontal spread of resistance genes. In order to overcome this issue, the new lysis plasmid was constructed by utilizing the approach of balanced-lethal systems based on auxotrophic gene Aspartate semialdehyde dehydrogenase (asd). The PhiX174 lysis gene E and λPR37-cI857 temperature-sensitive regulatory system was cloned in the asd gene positive plasmid and this novel approach allowed the production of antibiotic resistance marker free S. Enteritidis ghost. Although the generation of the S. Enteritidis was successful by using the above mentioned approach, but the unwanted leaky expression of lysis gene E occurred in the absence of induction temperature, and the bacterial host cell death during the normal growth condition at 28°C. To avoid leaky expression of the bacterial host-toxic PhiX174 lysis gene E from the λPR promoter, a convergent promoter construct was made in which gene E was placed between a sense λPR promoter and an anti-sense ParaBAD promoter. In the presence of L-arabinose, leaky transcription of lysis gene E at 28°C from the sense λPR promoter was repressed by an anti-sense RNA simultaneously expressed from the ParaBAD promoter. The stringent repression of lysis gene E in the absence of induction temperature resulted into higher concentration of bacteria in culture suspension, and consequently higher and stable production of a S. Enteritidis ghost. The S. Enteritidis ghost produced by above described approaches was characterized using scanning and transmission electron microscopy to visualize the transmembrane tunnel structure and loss of cytoplasmic materials, respectively. The efficacy of the bacterial ghost as a vaccine candidate was evaluated in a chicken model. The chickens from all immunized groups showed significant increases in plasma IgG and intestinal secretory IgA levels. The lymphocyte proliferation response and $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ T cell subpopulations were also significantly increased in all immunized groups. The data indicate that both humoral and cell-mediated immune responses are robustly stimulated. Based on an examination of the protection efficacy measured by observations of gross lesions in the organs and bacterial recovery, the candidate vaccine can provide efficient protection against virulent challenge.

Biography

Chetan V Jawale is basically a Veterinarian, currently pursuing PhD in Veterinary Medicine at College of Veterinary Medicine, Chonbuk National University, South Korea. He completed his BVSc and A H (2002-2007) from Bombay Veterinary College, Mumbai, India, and MVSc in Animal Biotechnology (2007-2009) from Anand Agricultural University, Anand, India. During 2009-2010 he worked as junior scientist at Xcelris Genomics Center, Ahmadabad, India. His research primarily focuses on the development of the recombinant and genetically inactivated vaccines against the *Salmonella* and *E. coli* infections in the domestic animals.

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