Use of attenuated *Bordetella bronchiseptica* as live vaccines and vehicle for heterologous antigens

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**Introduction**

*Bordetella bronchiseptica* is a respiratory pathogen which causes infections in mammals and birds. Unlike many other bacteria, the *B. bronchiseptica* can efficiently colonies healthy ciliated respiratory mucosa and possess all of the known bacterial protein secretion systems which means it can secrete all type of foreign antigens. Therefore, this research is focus on the study of the *B. bronchiseptica* mutant function as a live mucosal vaccine and vector for delivering heterologous antigens to the respiratory tract.

**Materials and Methods**

A wild type *B. bronchiseptica* strain which was isolated from a pig with atrophic rhinitis in China was attenuated by deleting its aroA gene through allelic exchange using suicide plasmid. Based on the study of the aroA mutant, then a double mutant strain of *B. bronchiseptica* lacking aroA and Type III Secretion and a *B. bronchiseptica* aroA mutant expressing porcine circovirus type 2 major capsid protein(PCV2-MCP) were constructed respectively to allow the PCV2-MCP to integrate into the chromosomal DNA of *B. bronchiseptica* through allelic exchange. The virulence of the aroA mutant and the double mutant were detected in order to know the safety. The ability of the wild type and the two attenuated strains to colonise the respiratory tract of mice was compared in 4 weeks female BALB/C by infecting intranasally and performing viable counts on homogenates of lung, trachea and nasal cavity. For the purpose of detecting the immunizing effect, both of the mutants were used to immunize pigs intranasally and then upon challenge with wild Type *B. bronchiseptica*. Serum anti-PCV2-MCP antibody titres were detected in mice immunized with the *B. bronchiseptica* -MCP recombinant stain to make sure of the possibility of expressing of PCV2-MCP by *B. bronchiseptica*.

**Results**

1. The virulence of the aroA mutant and the double mutant were decreased about 10 times and 225 times respectively compared with the wild type strain.
2. The numbers of the two mutants decreased rapidly in mice after post-infection, especially the double mutant. The numbers of the three strains were cleared from the lungs completely by day 28, day 14 and day 7 respectively. The trachea and nasal cavity samples of the aroA mutant decreased gradually, which were of clear of bacteria by day 21 and day 28. The double mutant showed the same trend and were cleared by day 14 and day 21 respectively. However the wild type strain could still be detected in trachea and nasal cavity up to 56 days.
3. Immunized pigs produced a strong serum and mucosal antibody response to *B. bronchiseptica*. Upon challenge with wild Type *B. bronchiseptica*, 100% of the two mutants immunized pigs were protected from fatal challenge. The titers of IgA antibody immunized with the two mutants were detected and much higher than the inactivated vaccine, demonstrated that mucosal response plays an important role in the protection from the challenge.
4. Serum anti-PCV2-MCP antibody titres can be detected in mice immunized with the recombinant stain, demonstrated that the MCP can be expressed by the aroA mutant.

**Discussion**

In consideration of the requirement of efficient colonization when using *B. bronchiseptica* as a vector, the aroA mutant was chosen to be a vehicle for heterologous antigens for the advantage that it can still express virulence determinants, which are often critical targets for stimulating protective immune responses. The suicide plasmid used in the research was disappeared finally from the strain because of its inability of surviving in other bacteria. Therefore, the three strains mentioned above contain no foreign antibiotic genes or other selection markers, so they may be good candidates for the development of a live attenuated vaccine against *B. bronchiseptica* infections and a vector for heterologous antigens.