RESEARCH ABSTRACT

SWINE HEALTH

Title: Development of a non-antibiotic selection vector for a live vaccine against PWD, **NPB #07-006**

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Scientific Abstract: Live vaccines are the most effective and the most affordable treatment for some infectious diseases, and current development of live vaccines is based on antibiotics selection system. But application of vaccines carrying antibiotic resistance genes has caused major concerns in environment and medical practice. Consequently, live vaccines using antibiotic selection vectors have been deemed as undesirable, unacceptable or even banned in some countries. Therefore, a non-antibiotic selection system must be developed. To construct a non-antibiotic selection vector, we isolated and amplified the *rtlt* operon from genomic DNA of an *E. coli* strain C using PCR with a reverse and a forward primers which were specifically designed to contain an *Ase*I and a *Spe*I restriction sites, respectively. The amplified PCR products were purified and digested with *Ase*I and *Spe*I restriction enzymes, so was the vector pBR322. The digested *rtlt* operon and pBR322 was ligated together with T4 DNA ligase. The resultant plasmid was introduced into an *E. coli* competent cell. Colonies selected by 2B minimal medium were initially screened with PCR to verify the presence of the *rtlt* operon, and the positive colonies were confirmed by DNA sequencing. Only *E. coli* cells transformed with the pBR322/*rtl*-operon can grow in the 2B minimal medium which contains ribitol as the only carbon source, thus it provided a non-antibiotics select marker. This 'pBR322/*rtl*-operon' vector still carried the tetracycline resistance gene. We replaced the *tet* region with a fusion antigen from LT and STb, and constructed a non-antibiotic selection vector to express vaccine antigens. By transformation of a nonpathogenic but K88ac fimbrial porcine *E. coli* field isolate (8511), we developed a non-antibiotic selected living vaccine 8595 to protect young pigs against porcine post-weaning diarrhea. This non-antibiotic selection system can also be applied in live vaccine development against other infectious diseases.