Construction of a *Bordetella pertussis* strain expressing the Pneumococcal Surface Protein A

Castro JT[1], Rivillas CS[1], Akamatsu MA[2], Debrie AS[3], Bezerra MFB[2], Miyaji EN[1], Locht C[3], Mielcarek N[3], Soares-Schanoski A[1], Ho PL[2], Oliveira MLS[1]

[1]Centro de Biotecnologia, [2]Divisão de Desenvolvimento Industrial e Produção, Instituto Butantan, São Paulo, Brazil;[3]Center for Infection and Immunity of Lille, Institut Pasteur de Lille, Lille, France.

Streptococcus pneumoniae (pneumococcus) infections are important causes of death in children less than 5 years old, worldwide. Pneumococcal Surface protein A (PspA) is a candidate antigen for the composition of protein-based vaccines against *Streptococcus pneumoniae*. We have previously used expression systems based on *E. coli* and lactic acid bacteria for the production of PspA. Here we aimed to construct a recombinant *Bordetella pertussis* strain expressing the PspA antigen. The *pspA*4 gene was cloned into a suicide vector in fusion with the N-terminal sequence of the *B. pertussis* filamentous haemagglutinin gene (*fha44*) and flanked by the upstream and downstream sequences of the dermonecrotic toxin gene. The recombinant plasmid, which conferred resistance to gentamicin and sensibility to streptomycin, was used to transform *E. coli* SM10 λ pir. Clones of the *B. pertussis* NIH 137 vaccine strain were selected for natural resistance to streptomycin and nalidixic acid. Transformation was performed through the conjugation with the *E. coli* bearing the plasmid. Bacteria were plated in media containing gentamicin (Gm) for the selection of recombinant clones with a copy of the plasmid integrated into the genome. A second recombinant event was selected through plating in media containing streptomycin (Str). Clones were evaluated by PCR using *pspA*-specific primers. Several Gm resistant clones were obtained after the conjugation protocol. Four of them, selected at random, were submitted to PCR using *pspA*-specific primers and the results showed a single band with the expected size of 1155 bp. Gm resistant clones were them plated into Str to induce the second event of recombination. This step produced 43 StrR clones that were also evaluated by PCR. The 1155 bp PspA band was confirmed in all of them. PspA expression in *B pertussis* was confirmed by western blot. Correct localization of the construct into the *B. pertussis* genome is being evaluated by PCR and DNA sequencing.

Keywords: *Streptococcus pneumoniae,* PspA, *Bordetella pertussis*.

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