

Selection and characterization of attenuated mutants of the APEC strain BEN2908

Daniel B. Pavanelo^{1,2}, Letícia Beatriz Matter³, Charles M. Dozois², Fabiana Horn¹

¹ UFRGS - Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

² INRS - Institut Armand Frappier, Laval, Canada

³ URI - Universidade Regional Integrada do Alto Uruguai e das Missões, Santo Ângelo, RS, Brazil

Avian pathogenic *Escherichia coli* (APEC) cause both localized and systemic infections in poultry. The lungs are an important site of entry of bacteria into the bloodstream, and for this reason we have investigated if APEC strains are capable of invading non-phagocytic cells. Out of eight tested APEC, only BEN2908 was able to invade avian fibroblasts (CEC-32). This strain harbors the same known virulence genes as noninvasive strains, and it also express type-1 fimbriae, an important adhesin for APEC. In order to identify genes contributing to the invasive phenotype of strain BEN2908, we created a library of mutants using the Signature-Tagged Mutagenesis (STM) approach, which consists of the generation of random Tn-inserted mutants. In addition to generating the mutation, the Tn-insertion works as a barcode for the mutants, allowing their identification even when they are pooled. Twenty pools of ninety mutants each were tested for invasion in avian fibroblasts, and 68 single mutants seemed to be attenuated for invasion of fibroblasts. These mutants were tested for type-1 fimbriae expression by yeast agglutination, and three of them were unable to agglutinate yeast. This inability was confirmed by interaction and invasion assays, in which they failed to invade and had a ten-fold drop in interaction. One of these mutants has been sequenced and the Tn insertion was located in the *fimA* gene, the major type-1 fimbriae fimbrin. The other 65 mutants, positive for yeast agglutination, are being tested individually in interaction and invasion assays with fibroblasts. Out of 25 tested mutants, 3 are attenuated for interaction with, and 5 are attenuated for invasion of cells. The other 40 mutants will be tested, and the confirmed attenuated ones will be sequenced. The results of this work will allow us to better understand not only the invasive phenotype of the APEC strain BEN2908, but also mechanisms of regulation in virulence of APEC strains and other pathogenic *E. coli*.