TITLE: Identification of pathogenic avian Escherichia coli genes involved in invasion of avian fibroblasts by Signature Tagged Mutagenesis (STM)

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ABSTRACT:

The strain MT78 is an E. *coli* capable of causing extraintestinal infections so it is classified as an ExPEC. It invades nonphagocytic avian cells and may have genes that influence its virulence that have not yet been described. Looking for genes that can influence the virulence of this strain, through the technique of STM (signature-tagged mutagenesis) a library of 1710 random mutants of the MT78 was created, those with attenuated invasion to non-phagocytic cells were selected. Among the attenuated mutants found, one lost the gene for the enzyme phospho-β-glycosidase B (*bglB* gene, which belongs to the *bgl* operon).

Adhesion and invasion of the *bgl* operon mutant were tested on avian fibroblasts of the CEC-32 lineage. The mutant's adhesion capacity was found to be reduced to less than half the wild strain capacity (45%), while the invasiveness was reduced to 32% of the wild strain capacity. Beyond cell testing, in vivo tests were performed where a 10 fold reduction in the bladder colonization capacity was observed in mice.

For the complementation of the MT78Δbgl mutant, the bgl operon will be amplified from the wild-type MT78 DNA. This operon, however, has 8.3 kb of extension, which makes difficult its manipulation. To facilitate its cloning into a vector, the operon was divided into 4 separate sections of approximately 2 kb having complementary bases at each other ends. In a single first PCR these sections will be amplified individually; Afterwards, will be mixed and the whole operon will be amplified in a second reaction.

After amplification the operon will be cloned in a DH5αλpir strain using as vector the pGPTn7-cm plasmid. The plasmid pGPTn7-cm containing the *bgl* operon will be extracted and the MGN-617ΔDAP strain will be transformed with it, and then conjugated with the mutant strain MT78Δbgl containing the plasmid pSTNSK. MGN-617ΔDAP will donate the plasmid pGPTn7*bgl*-cm and the transposases encoded in plasmid pSTNSK will insert the bgl operon and a chloramphenicol resistance cassette in the att region of the mutant chromosome. This complementation method is advantageous because the complemented mutant has only one copy of the inserted gene and does not depend on the presence of antibiotic for its replication, as occurs in plasmid complementations.

The complemented mutant will then be subjected to avian cell adhesion and invasion assays as well as in vivo tests to confirm the wild type phenotype recovery.