Title: THE INFLUENCE OF THREALOSE METABOLISM ON TYPE I FIMBRIA EXPRESSION IN EXTRAINTESTINAL *E. COLI* MT78

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) are responsible for a number of infections outside the gastrointestinal tract, including urinary tract infections and neonatal meningitis in humans, and infections in farmed animals. In poultry, avian colibacillosis – as ExPEC infections are collectively called – causes great economic losses worldwide. After screening a library of 1710 mutants of the ExPEC MT78 strain generated by signature-tagged mutagenesis, one of the attenuated mutants had the *treA* gene affected. This mutant showed a decrease in type I fimbriae expression, a reduction of 35% in adhesion and of 65% in invasion of avian fibroblasts (CEC-32 cell line) compared to the wild type strain. The enzyme trehalase, encoded by the *treA* gene, degrades the disaccharide trehalose in the periplasm; *otsA* and *otsB* genes encode the cytoplasmic enzymes of trehalose biosynthesis. In *E. coli* K-12, the pathways of trehalose biosynthesis and degradation are involved in the response to osmotic stress. Thus, under osmotic stress, K-12 accumulates trehalose by increasing the expression of *treA, otsA* and *otsB*. In order to elucidate whether the excess of trehalose in the periplasm is affecting type I fimbriae expression, we generated mutants MT78Δ*otsAB* and MT78Δ*treAotsAB* by the non-polar mutation lambda red technique. Afterwards, we performed yeast agglutination tests to observe type I fimbriae expression in these mutants. Bacterial cultures were grown both statically for 24 hours and under shaking up to the mid-log phase (OD 600 nm ~ 0.6) in LB medium. An initial suspension of approximately 10^{11} CFU in PBS was serially diluted 1:2 in microtiter plates. Equal volumes of a 1.5% yeast solution were added to the wells. After 30 minutes on ice, the agglutination was visually monitored and the most diluted titer with agglutination was determined. Strain DM34, a type I fimbria null mutant of MT78 was used as a negative control. The most diluted yeast agglutination titers were: 2^8 for wild-type strain, 2^6 for MT78Δ*otsAB* and 2^3 for MT78Δ*treAotsAB*. Our results indicate that excess of trehalose in the bacterial periplasm is not responsible for the observed reduction in type I fimbriae expression; this reduction may be explained by other mechanism yet to be elucidated.

Key words: *E.coli* MT78, *otsA, otsB, treA*, type I fimbriae.

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