TITLE: ANALYSIS OF THE ACTION OF LYSINE DECARBOXYLASE OPERON IN AN AVIAN PATHOGENIC ESCHERICHIA COLI (APEC) STRAIN ISOLATED FROM A SWOLLEN HEAD SYNDROME CASE.

AUTHORS: VITORINO, M.T.; GARBIM, J.L., MALUTA, R.P.; ROJAS, T.G.; ZUPIROLLI, B.; DIAS da SILVEIRA, W.

INSTITUTION: UNIVERSITY OF CAMPINAS, CAMPINAS, SP (AVENIDA CÂNDIDO RANDON, 400, CEP 13.083.875, CAMPINAS – SP, BRAZIL)

ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) strains are responsible for several infectious processes in poultry. In some pathogenic *E. coli* strains of human origin the operon cad [(constituted by three genes, *cadC* (regulator gene), *cadB* (encoding an anti-transporter for lysine decarboxylase – cadaverine) and *cadA* (induces lysine decarboxylase)], responsible for the decarboxylation of lysine, is related to pathogenicity characteristics. The final objective of this work is to verify the role of operon cad (Lysine Decarboxylase) in biological characteristics and pathogenicity of an APEC strain (SCI07) isolated from a chicken presenting clinical signs of Swollen Head Syndrome. The pkD46 plasmid hosted in Escherichia coli strain DH10β and pKD3 plasmid hosted in Escherichia coli strain S17λpir were extracted. Transformation of SCI-07 strain and selection of transformants was performed. The electroporation of the insert was made followed by the selection of mutants. The selection was performed by PCR to confirm homologous recombination between the antibiotic resistance cassette and the target gene. Strains were evaluated for their ability to adhere to human epithelial cell line in the presence and absence of the D-mannose analog. Cultures of each strain grown overnight in LB broth were stabbed into the center of LB 0.3% agar plates, the plates were incubated at 37°C and motility was measured after 10h. In vivo mortality test, assessment of mortality was held in one-day-old, broiler chickens briefly, groups of 20 birds for each strain were inoculated in the right thoracic air sac with 0.1ml of a bacterial suspension containing 10^9 CFU or with 0.1ml. Birds were observed every 12h up to 07 days after inoculation and the number of deaths. The systemic infection experiments were performed the group of 7 one-day-old chickens for each strain as infected with a bacterial suspension containing 10^7 CFU/ml, in the right thoracic air sac. At 24 and 48 hpi, chickens were euthanized. The three desired mutants were obtained, the mutants of each gene were constructed separately of cad operon, for biofilm formation, we observed differences of growth between the wild-type, mutants and complemented strains. For adhesion, the mutants had greater capacity than the other strains, different from the invasion assay, where the wild strain was more efficient. In the in vivo assays, it was possible to conclude that without the operon there are fewer deaths and less infected organs.

Keywords: APEC, operon cad, lysine decarboxylase, mutants, lambda red.

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