AMYLOID PROTEINS PRODUCED BY *GALLIBACTERIUM ANATIS*

Lopez Ochoa AJ[1], Negrete Abascal E[2], Vaca S[2], Soriano E[3], Sanchez Alonso MP[1] & Vazquez Cruz C[1]. [1]Centro de Investigaciones en Ciencias Microbiológicas, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla Pue. CP 72570, [2]Carrera de Biología, Facultad de Estudios Superiores Iztacala UNAM. [3]Facultad de Medicina Veterinaria y Zootecnia UAEM. Email:ecobacilos@yahoo.com

Key words: Gallibacterium anatis, Curli, Amyloid proteins

Introduction. *Gallibacterium anatis* is the bacterial pathogen causative of gallibacteriosis in chicken, a complex illness that upsets poultry economy in several countries. Recently it has been hypothesized the production of amyloid proteins during biofilm formation by this bacteria is a virulence factor. In the well-studied model system enteropathogenic *E. coli,* curli production has been depicted as an important piece to construct the biofilm structure and to attach the bacteria to intestinal cells. In *E. coli* and *Salmonella* curli´s genes are arranged in two divergent operons; in one operon the *csgA-csgB* genes are transcribed under regulation of the products of *csgD* gene, which is encoded and transcribed in the opposite direction together with *csgE-csgF-csgG*. *G. anatis* produces biofilm but we do not know if this bacteria produces amyloid proteins as *E. coli* does.
Despite both bacteria belongs to different taxonomic groups, here we sought for amyloid-protein production by *G. anatis* to explore its use as target for gallibacteriosis control.

Methods. A strains collection of *G. anatis* was cultured in conditions to enhance biofilm production. Congo red dye (CRD) was added to the cultures to stain bacteria. Also resistance to boiling with formic acid was performed to find potential amyloid proteins. Proteins from culture supernatants and from the whole cell extracts (WCE) were analyzed by SDS-PAGE. Western blotting of the stained proteins was performed in order to detect curli related proteins using anti-curli polyclonal antibodies. Search for curli genes was attempted by PCR using oligonucleotide primers for *E. coli* curli protein, and by DNA-DNA hybridization.

Results. Reference strains F149 and 12656-12 of *G. anatis* and 14 field isolates were cultured with CRD. It was found that all tested strains bind the dye as a general trait. When compared concentration of CRD-stained proteins in whole cell extracts against soluble proteins in supernatant, a higher concentration was found in the former source, whereas, several proteins were found at similar concentrations in the two protein pools. Western blot with anti-curli was positive for reference strains. Also parallel assays using medium added with, or without CRD discarded protein induction by the dye. Proteins resistant to formic acid were observed in several strains: four strains (25%) produced four protein bands and two (12.5%) showed one intense protein band, remaining strains (62.5%) don not accumulate curli like proteins. The assay was performed in supernatants and WCE of bacterial cultures growing in presence or absence of CRD and the result was similar in both conditions. PCR amplification of curli genes was negative in all strains, as well as the southern blot hybridization assays when used *E. coli csgG* and *csgA* genes encoding curli. Positive hybridization was only observed for a recently *csgG* gene proposed for *G. anatis.*

Table 1. Protein amyloid and curli related genes in bacterial collection of *G. anatis*.

Condition	Strains positives	Strains negatives
Four band of amyloid proteins	4 (25%)	12 (75%)
csg presence	16 (100%)	0
csg A – csgB presence	0	16 (100%)

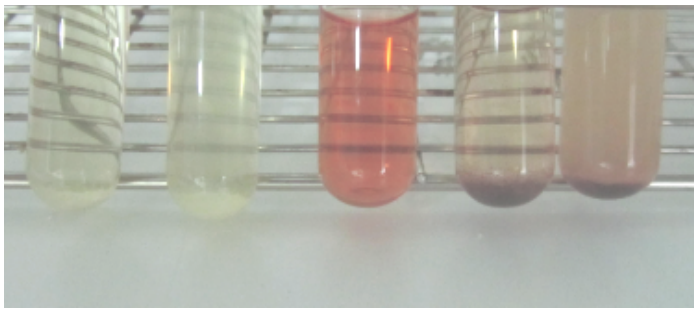

Fig.1 *Gallibacteium anatis* growth to search amyloid proteins. Tubes arranged from left to right: first medium without growth, second medium with growth, third medium containing Congo red without growth, fourth medium with growth in Congo red and shaked condition and fifth medium with growth in Congo red and static condition.

Conclusions. *G. anatis* produces several amyloid proteins but the majority curli genes are absent, then curli proteins are encoded by genes different to that known in *E. coli* and *Salmonella.* The curli associated gene *csgG* is the only resident in *G. anatis* suggesting its critical conservation for amyloid protein transport.

Acknowledgements. The project was partially supported by ICUAP-BUAP and PAPIIT IN216010 UNAM.

References.

1. Vaca S, Monroy E, Rojas L, Vazquez C, Sanchez P, Soriano E, Bojensen M, Negrete E. (2011). J. Animal, Vet Adv. 10(13): 1688-1693.
2. Salgado M, Vaca S, Vazquez C, Zenteno E, Rea I, Perez V, Negrete E. (2012). Advances in Microbiology 2: 505-510.
3. Johnson TJ, Danzeisen JL, Trampel D, Nolan LK, Seemann T, et al. (2013). PLoS ONE 8(1): e54844. doi:10.1371/journal.pone.0054844.