Inactivation of *pbp4b* gen en *Escherichia coli* BW 25113, CS-802-2, RP1 and *mraZ* y *mraW* genes of *dcw* cluster in *Salmonella enterica serovar* Typhimurium SL1344.

Vega Mendoza D.E * y Ayala Serrano J.A
*Centro de Biología Molecular Severo Ochoa de Madrid- Universidad Autónoma de Madrid-Campus Cantoblanco-28049 *dvega@cbm.uam.es*

At the present time, the synthesis of new artificial antibiotics more effective as well as the discovery and improve of already existing antibiotics, suppose an authentic medical revolution in the treatment of infectious diseases. Nevertheless, the versatility and adaptability extreme of the micro organisms have avoided that the human victory on the pathogenic bacteria has been total; many bacteria have been developing throughout history mechanisms that protect them in front of many antibiotics.

Therefore we are trying to focus this very important problematic through a strategy that allows to understand what are the molecular mechanisms (and the proteins implied in them) by which *Salmonella enterica serovar* Typhimurium SL1344 (STM) carries out the cellular division and Peptidoglycan biosynthesis within the cell eukaryotic, and that contribute to the keys for the design of therapeutic strategies that can limit the infection and that would prevent the appearance of antimicrobial resistance, using like model organism several strain of Escherichia coli.

Since the pathogenicity of STM comes given by its capacity to accede to non phagocitics host cells, we have designed a mutant of STM and e. coli for the *pbp4b* gene implied in the process of cellular division and the Peptidoglycan biosynthesis.

Focusing the cellular division process, and specifically the genes *mraZ* and *mraW* of dcw cluster, we are trying to understanding the molecular mechanisms, and proteins implied in them, by which STM divides within the eukaryotic cell, being able these new data, to contribute keys for the design of therapeutic strategies that can limit the infection and that would prevent the appearance of resistance

This mutants were obtained by a new technique of PCR, using primers of 60 nt. of length, which amplify the cassettes of chloramphenicol and kanamycin resistance that are flanked by regions FRT (FLP Recognition Target) and homologous regions to adjacent genes to the target genes and then transform bacterial strain containing the pKD46 vector of low number of copy and temperature-sensible that codifies for the Red recombinase of the Lambda phage that is under the control of an inducible promoter by arabinosa, and the mutants generated were selected by their resistance to antibiotic. These resistance genes were eliminated using a vector temperature-sensible that codifies for FLP recombinase.

This mutants have been put under studies of kinetic of intracellular proliferation using mice model as well as in vitro tests using cellular lines HeLa, Swiss 3T3 and NRK for the obtaining of the respective rate of intracellular proliferation in non phagocytics cells.

The results obtained until the moment indicate that STM displays a different infection behavior and intracellular proliferation depending on the cellular type that it invades.

In the case of the mutant of E. coli, CS-802-2, it presents deleted the genes that codify for PBP1a, 4, 5, 6, 7, AmpC, AmpH and DacD. The main objective is get the inactivation and disruption of the *pbp4b* gene and determine if the function of the PBP4b is essential in vivo. The results obtained show the non essentiality of this protein.