VACCINE DEVELOPMENT USING LIVE *SALMONELLA ENTERICA* SV TYPHIMURIUM STRAINS DELETED IN FLAGELLIN -ENCODING GENES USING LAMBDA RED RECOMBINATION APPROACH

Massis, L.M.[1,2]; Newton, S.M.C.[2]; Sbrogio-Almeida, M.E.[3], Klebba, P.E.C.[2]; Ferreira, L.C.S.[1]

1-Departamento de Microbiologia-ICBII, USP, São Paulo, Brazil; 2- Department of Chemistry-Biochemistry, University of Oklahoma, USA; 3- Instituto Butantan, São Paulo, Brazil

Salmonella flagellins are potential carriers of vaccine antigens. Nonetheless, this system requires a flagellin-deficient strain that can produce recombinant flagellins fused to heterologous epitopes. *S*. Typhimurium has two non-allelic flagellin genes, *fliC* and *fljB*, which encode antigenically distinct proteins and the *fljA* gene wich downregulates the *fliCi* expression. Thus, the objective of the present study was the construction of *S*. Typhimurium strains deleted in the different flagellar genes. A non-flagellated strain was constructed using a site-specific recombination with a linear DNA fragment using the Red recombination system. Linear DNA fragments were amplified by PCR with downstream and upstream regions of the genes to be deleted (*fliCi*, *fljBA*) and an antibiotic resistance marker. The site-specific recombination was obtained after transformation of a *S.* Typhimurium strain carrying a temperature-sensitive replication plasmid encoding the expression of the ? Red. The deletions were confirmed by PCR, motility tests and immunoblots with specific anti-flagellin antibody. This technique allowed the selections of stable *S.* Typhimurium mutants unable to express one or both flagellar antigens. The availability of strains defective in flagellin expression represents an important tool for the development of attenuated *Salmonella* strains that express recombinant flagellins fused to heterologous epitopes.

Supported by: FAPESP