P81

Contribution of antigenicity by Lpp, a *Salmonella* surface antigen in the immune response

Jong Earn Yu, Young Hee Kim, Seong Kug Eo[1],
John Hwa Lee[1] and Ho Young Kang

Department of Microbiology, Division of Biological Science, College of Natural Science, Pusan National University, Busan 609-735, Korea
[1]School of Veterinary Medicine, ChonBuk National University, JeonBuk, 561-756, Korea.

Although, several immunization of attenuated *Salmonella* live vaccine induce strong protective immune responses, it has not been identified the *Salmonella* major antigens which contribute to induce strong immune responses. To identify immunodominant *Salmonella* antigens, attenuated *S. typhimurium*(Δ*crp*) live vaccine was administrated into BALB/c mouse with a single 1 X 10^9 CFU dose through the oral route. The sera collected from immunized mice were used to detect the antigens in *S. typhimurium* cell lysates by immunoblot assay. An immuno-reactive protein band was detected at approximately 6.9 kDa. The protein purified from outer membrane fraction of *Salmonella* was analysed to identify the protein through a MALDI-TOF assay system. The protein was determined as Lpp which is major bacterial outer membrane lipoprotein component of Gram negative bacteria in the family of *Enterobacteriaceae*. The 5′-flanking and 3′-flanking regions of *lpp* gene were amplified by PCR, joined and cloned into a suicide plasmid, resulting in a recombinant suicide plasmid pBP109. A *S. typhimurium* mutant deleting *lpp* gene was constructed by allelic exchange with recombinant suicide plasmid pBP109, resulting in *S. typhimurium* CK23. The *lpp* gene deletion in CK23 was verified by DNA size comparison of PCR amplified DNA fragment of *lpp* region. Additional confirmation was performed by elimination of 6.9 kDa immuno-reactive protein in immunoblot analysis. Lpp-specific polyclonal antibody was produced in a New Zealand White rabbit. The antibody production was determined by immunoblot with sera obtained from primary immunization. With the use of prepared anti-Lpp antibody an immuno-reactive 6.9 kDa protein band was detected in wild-type χ3339 strain but it was not detected in CK23, indicating that the polyclonal antibody is Lpp specific.