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Omp31 plays an important role on outer membrane properties and intracellular survival of *Brucella melitensis* in murine macrophages and HeLa cells

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 $B_{\rm all}$ species of mammals, including humans, and is a major zoonosis worldwide. Brucella sp. are facultative intracellular pathogens that have the ability to survive and multiply in phagocytic and non-phagocytic cells such as trophoblast and epithelial cells. Among the six recognized species of the genus Brucella, Brucella melitensis is the main etiological agent involved in goat brucellosis and is also the most pathogenic for human. It causes significant losses in livestock production as a result of abortions, metritis, infertility, and birth of weak animals. Outer membrane proteins (OMPs) are exposed on the bacterial surface and are in contact with cells and effectors of the host immune response, whereby they could be important virulence factors of Brucella species. To evaluate this hypothesis, the gene encoding for the major outer membrane protein Omp31 was amplified, cloned into pUC18 plasmid, and inactivated by inserting a kanamycin cassette, rendering pLVM31 plasmid which was transformed into B. melitensis wild-type strain to obtain LVM31 mutant strain. The outer membrane (OM) properties of the mutant strain were compared with B. melitensis Bm133 wild-type and B. melitensis Rev1 vaccine strains, in assessing its susceptibility to polymyxin B, sodium deoxycholate, and

nonimmune serum. The mutant strain was assessed *in vitro* with survival assays in murine macrophages J774.A1 and HeLa cells. Our results demonstrate that LVM31 mutant is more susceptible to polymyxin B, sodium deoxycholate, and nonimmune serum than control strains. Moreover, Omp31 mutation caused a decrease in the internalization and a significant decrease in the intracellular survival compared with the reference strains in both cell lines. These results allow us to conclude that Omp31 is important for maintaining OM integrity, but also it is necessary for bacterial internalization, establishment and development of an optimal replication niche, and essential for survival and intracellular multiplication.

Speaker Biography

Lázaro Verdiguel-Fernández has completed his Master's degree from National Autonomous University of Mexico. Currently, he is a PhD student. He is Professor of Veterinary Immunology and Applied Molecular Microbiology and directed five undergraduate thesis. He has published one paper in *Archives of Microbiology Journal*. He has participated in the International Brucellosis Research Conference including the "69th Annual Brucellosis Research Meeting", New Delhi, India, 2016. He is a member of the Biotechnology Committee of the National Technical Advisory Council on Animal Health of Mexico.

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