Abstract

Shiga Toxin producing *Escherichia coli* (STEC) strains are highly zoonotic foodborne pathogens. These represent a serious problem for public health, being the main responsible for the disease known as Hemolytic Uremic Syndrome (HUS), endemic in Argentina.

The molecular bases of the infection mechanism are complex, involving several virulence factors. The proteins encoded by the Locus of Enterocytes Effacement (LEE) have been tested as components of vaccine formulations against carriage of STEC in cattle. This locus includes EspA and EspB, and the translocation receptor for Intimin (Tir), which are secreted through a type III secretion system (TTSS), and the adhesin Intimin.

In this thesis work, a tool was developed for the analysis of the expression of recombinant proteins, applicable to others of interest. This tool is useful for evaluating the antigenic composition of vaccine formulations and for quality control of their production processes, among others.

We worked with three recombinant virulence factors: EspA, Intimin and the B subunit of the Shiga Toxin 2 (Stx2B). This protein plays a main role in the colonization of cattle and is responsible for pathogenicity in humans.

In the first part of this work, the expression and purification conditions of the recombinant proteins were optimized, and polyclonal sera were generated aimed at their specific recognition.

Based on the limitations observed, in the second part, a different strategy was addressed by developing an analytical tool based on liquid chromatography (LC) coupled to Mass Spectrometry (MS).

LC separation conditions were optimized and, within MS-based techniques, we worked on the quantification of proteins in their complete structure using multiple reaction monitoring (MRM). An automated optimization of all the variables tested was carried out through the Labsolutions software provided by the Shimadzu LC-MS 8030 equipment. From these optimized conditions, a calibration curve was carried out on samples containing known quantities of proteins of interest, contrasting the signal intensity in the MRMs with the protein concentration in the sample. This work provides a detection/quantification methodology that is applicable for more complex samples and as a control tool in a national reference laboratory such as the R&D&i within the National Institute of Biological Production of the ANLIS “Dr. Carlos G. Malbrán”.

Keywords: HUS, recombinant protein, mass spectrometry, quantification.