Isolation of Escherichia coli O157 Proteins that Interact with the Bovine Recto-anal Junction Squamous Epithelial (RSE) Cells

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The enterohemorrhagic strain Escherichia coli O157:H7 ranks fourth among the agents causing gastrointestinal illnesses in North America. Cattle are the main reservoirs for this human pathogen with the Recto-anal junction (RAJ) serving as the primary colonization site. The objective of this study is to examine the mechanism by which E. coli O157 adheres to the squamous epithelial (RSE) cells at the bovine recto-anal junction. An adapted protocol was developed to isolate the E. coli O157 bacterial proteins that interact with the RSE cell membrane proteins. After checking the RSE cells for good morphology, the RSE cell surface proteins were extracted and labeled using the Thermo Scientific Pierce cell surface protein isolation kit. The E.coli O157 proteins were harvested and interacted with the RSE cell surface proteins in a biotin cell protein column. The interacting bacterial proteins were eluted and then quantitated using NanoDrop. These proteins are currently sent for bottom-up proteomics analysis. A follow up experiment, an adherence-inhibition assay, was conducted and the results clearly showed that the interacting bacterial proteins isolated in this experiment play a role in E. coli O157 adherence to the RSE cells at the RAJ.

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