Microsc. Microanal. 26 (Suppl 1), 2020

doi:10.1017/S1431927620001129

The Role of Glucose in the Pathology of EHEC O157: H7

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The pathogen enterohemorrhagic *Escherichia coli* (EHEC) O157: H7 is responsible for hemorrhagic colitis and hemolytic uremic syndrome in humans [1]. During the colonization process in the gastrointestinal tract, EHEC needs to adapt to changes in nutrient availability [2]. The objective of this study was to evaluate the influence of glucose on physiology and processes involved in the pathogenesis of EHEC O157: H7 in order to improve our understanding of the mechanisms controlling EHEC growth and survival in the bovine gut.

In this study we first analyzed the growth rate of EHEC O157: H7 Rafaela II clade 8, a strain isolated from a bovine in Argentina, grown in the medium DMEM supplemented with either 4.5% glucose (High-glucose - DHG) or 1% glucose (Low-glucose - DLG). In addition, we assessed the bacterial adhesion capacity and actin pedestal formation induced by EHEC [3] by performing infection assays. For this purpose, Caco-2 epithelial cells were exposed for 5 h with Rafaela II grown with the different concentrations of glucose. Subsequently, the samples were fixed (paraformaldehyde 4%) and permeabilized (triton); actin and nucleic acids (DNA) were stained with rhodamine-phalloidin and TO-PRO-3, respectively. Bacterial adhesion capacity and pedestal formation of cells were evaluated using a Leica TCS SP5 laser scanning confocal microscope (MC). Each Image was acquired by monitoring a single focal plane over time (xyt scanning mode) using a 40X/1.25 oil objective lens and 543nm HeNe and 633 nm HeNe lasers. The frequency and resolution for acquiring images were set at 200 Hz and 1,024 x 1,024 pixels, while maintaining the same settings for laser powers, gain, and offset.

The growth rate of Rafaela II was similar under either condition (DHG and DLG). According to the MC observations of the infection assays, however, Rafaela II grown in DLG displayed smaller cell size as well as greater ability to adhere to Caco-2. Furthermore, the cells infected with the strain grown in DLG presented higher actin accumulation. This actin rearrangement is consistent with the formation of pedestals and is characteristic of the “attaching and effacing” lesions that contribute to the diarrheal manifestations caused by EHEC infection [4].

These preliminary assays demonstrate that glucose plays a role in processes related to EHEC physiology and pathogenesis of EHEC O157: H7 Rafaela II clade 8.

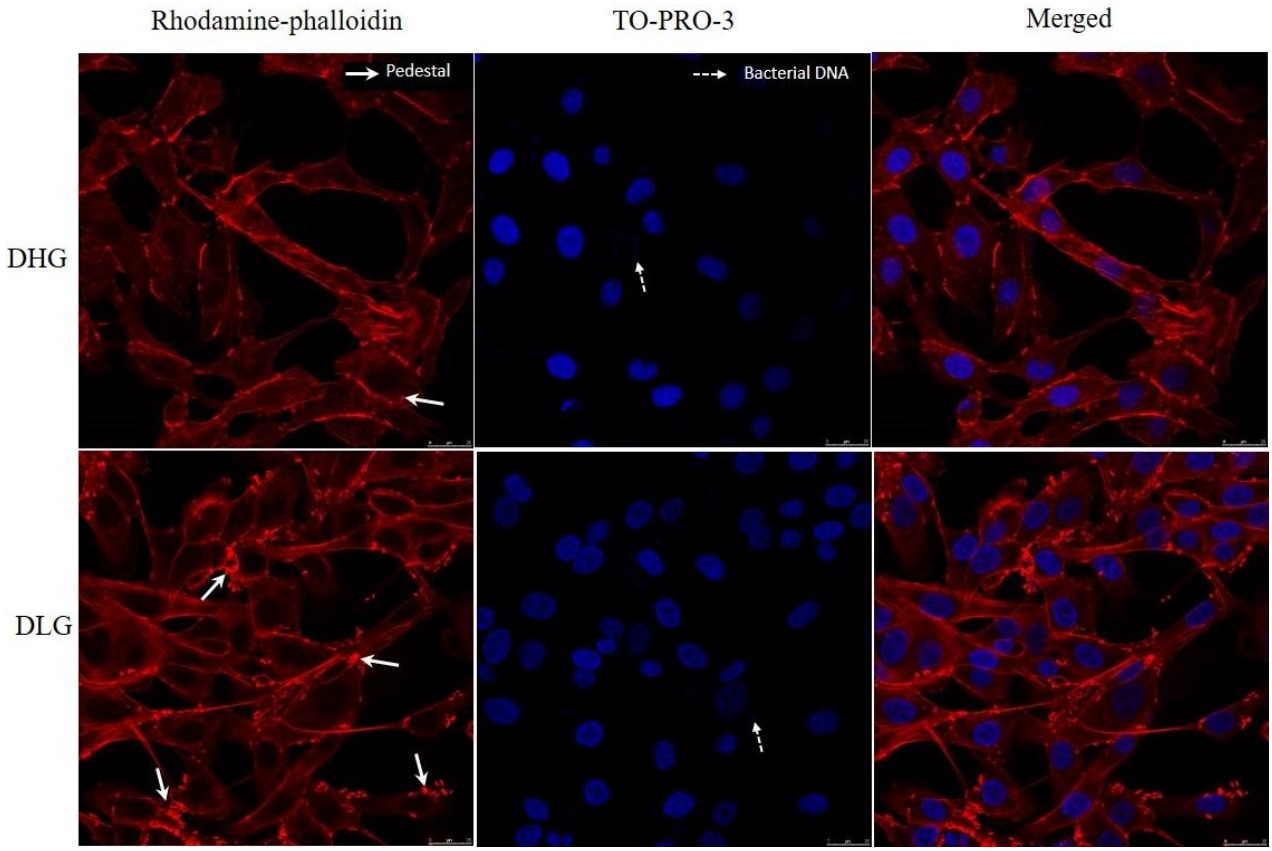

Figure: Actin pedestal formation in Caco-2 cells infected with Rafaela II. Confocal images of Caco-2 cells infected with Rafaela II grown in DMEM supplemented with 4.5% (DHG) or 1% (DLG) glucose. The cells were fixed (paraformaldehyde 4%) and permeabilized (triton). Subsequently, DNA and actin were labeled with TO-PRO-3 (blue) or rhodamine-phalloidin (red), respectively. The images were acquired by a Leica Sp5 confocal microscope (40X/1.25AN oil lens; 200 Hz; 1,024x1,024 pixels) using HeNe 543 and HeNe 633 lasers. The same settings for laser powers, gain, and offset were maintained between DHG and DLG. Infections with Rafaela II grown in the DLG condition displayed higher pedestal formation. Complete arrows indicate actin accumulation (actin pedestal formation), whereas dashed arrows show bacterial DNA.

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https://doi.org/10.1017/S1431927620001129