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Title of thesis: The Effects of Postbiotics from *Lactobacillus acidophilus* LA-5 on Intestinal Epithelial Cell (HCT-116)

Abstract

The use of probiotics in humans and food has always been associated with challenges such as shelf life, stability and limitation of use. Postbiotics (Pb) as the metabolites of microbial cultures have gained increasing attention due to beneficial effects, which can effectively address the issues associated with the use of probiotics. The purpose of this study is to prepare two types of postbiotic from *Lactobacillus acidophilus* LA-5 in classical culture medium (De Man, Rogosa and Sharpe agar; MRS- Pb) and whey solution (W- Pb) aiming to compare their effects on HCT-116 cells as intestinal epithelial cancer cell line. MTT test was used to evaluate the cytotoxic effects of postbiotics in concentrations of 0.625, 1.25, 2.5, 5, 10, 20 and 40 mg/mL and the trypan blue test was used to determine the living cells. The results of the MTT test indicated a significant decrease in the viability of HCT-116 cells treated with postbiotics in a concentration-dependent manner, so that high concentrations (10, 20 and 40 mg/mL for MRS- Pb and 10 and 20 mg/ml for W-Pb) have caused a significant decrease in the survival rate of HCT-116 cells through a decrease in mitochondrial activity or mitochondrial regeneration capacity in cells. Also, MRS-Pb has exhibited a more level of changes and cytotoxicity compared to W-Pb. The wound healing assay was used to evaluate the amount of cell migration and proliferation. It is noteworthy that neither of the postbiotics demonstrated a significant impact on cell proliferation and migration. Furthermore, oxidative and antioxidative status of the postbiotics was determined by total oxidant status (TOS) and total antioxidant capacity (TAC) in the supernatant from the treated cells. The results obtained indicated that the antioxidant capacity of both groups of cells, when exposed to increasing concentrations of postbiotics, was significantly enhanced in a manner that was dependent on the concentration. It should be noted that the oxidant status of both groups at 10 mg/mL concentration increased, however, given the morphology of the cells and the results from the cytotoxicity assay, this increase is negligible. By comparing the results from TAC and TOS it can be concluded that the MRS-Pb has more antioxidant performance than the W-Pb, but no significant difference was observed in their oxidant properties. Considering the results of the present study and previous data, it seems that postbiotics can be a potential candidate to serve as an alternative for probiotics without their associated limitations, to be used in the production of nutraceuticals. The study also investigated the use of whey as an alternative to MRS and found that whey does not have the unpleasant color associated with bacterial culture media, making it a viable option for commercial use of postbiotics in the food, feed, and drug industry.

Keywords: Postbiotics, Lactic acid bacteria, Probiotics, Colon cancer, Whey solution.