Chapter 1

Introduction

1.1 Background

A certain type of bacteria called the lactic acid bacteria (LAB), has a distinctive property that can adapt to various conditions, especially withstanding low pH environments, and can immediately change its metabolism accordingly (George et al., 2018). LAB are generally used for food fermentation processes, however, research on this type of bacteria has led to the development of these bacteria as probiotics because of their ability to attach to abiotic or biological surfaces, able to survive, and have metabolic activity in the intestinal environment while being non-pathogenic, and inhibit the growth of intestinal pathogens; possess antimicrobial activity with bacteriocin production (Andreevskaya et al., 2018; Dowdell et al., 2020).

According to Shi et al. (2016), when a group of bacteria improves or restores the balance of the gut microbiota, they can be referred to as probiotics. Most of them are consumed to prevent bowel diseases, boost the immune system, as well as for the balance of gut microbes (Wang et al., 2021). In the last decade, LAB has been investigated as a good probiotic because it can produce bacteriocin while adhering to gut epithelial cells. Bacteriocin is a secondary metabolite product with antimicrobial properties and works by creating pores on the cell surface, increasing cell permeability, and decreasing the formation of a cell wall, thereby affecting nucleic acid synthesis, resulting in the inhibition of protein synthesis (Kumariya et al., 2019).

The adhesion ability of probiotics to the human intestinal cells is also one of the important properties to become a good probiotic. The probiotics' mode of action in adhering to the human intestinal epithelial cells is driven by the presence of surface proteins such as the mucus and pili binding proteins, fibronectin-binding proteins (FBPs), and surface layer proteins (SLPs). With these surface proteins, they help to enhance hydrophobicity and adhesion of the probiotics to the intestinal cell walls. Therefore, if probiotics possess this ability, they will inhibit pathogen adherence by competing

for host cell binding sites, preventing adhesion (Monteagudo-Mera et al., 2019). However, there is currently a lack of understanding of the function of probiotics in binding to human intestinal cells due to insufficient data leading to failure in defining the importance of probiotics (Khan et al., 2019). Therefore, in order to define the function of probiotics, a deeper comprehension of this ecosystem is required notably for the purpose of managing pathogenesis through the use of probiotics as therapeutic agents.

In order to determine the activity of probiotics in human intestinal cells, an *in-vitro* method can be used using human epithelial cells from colon carcinoma namely the Caco-2 cell line. Because of its capacity to develop into monolayer cells, this human epithelial cell line is frequently utilized as a model for the intestinal epithelial barrier with various properties found in the small intestine (Lea, 2015). In addition, the intestinal mucosal barrier characteristics' especially on the biological and biochemical underpinnings can be learned from the Caco-2 cell line so that it will be very useful in studying the mechanisms and effects of probiotics especially in adhering to human intestinal cells while also being harmless which would be the goal of this project (Shimizu, 2010). Finally, this project is anticipated to solve the information gap about how probiotic bacteria safely affect the colonization of the gastrointestinal system and counteract the toxicity of food-borne pathogens by an *in-vitro* method utilizing the Caco-2 cell line.

1.2 Objective

The objective of this research are; to elucidate the properties and/or investigate the ability of probiotics in adhering to the human colon adenocarcinoma cell line (Caco-2 cell) and observe the effect and safety of their secondary metabolites to the cell with the presence of pathogenic bacteria. The outcomes will offer crucial knowledge on using these bacteria as probiotics on counteracting food-borne pathogens.

1.3 Hypothesis

E. faecium and *L. lactis* have the properties required as a good probiotic which is able to adhere effectively to intestinal epithelial cells. Furthermore, the metabolites obtained from both lactic acid bacteria in the form of supernatant will provide a protective effect to Caco-2 cells against food-borne pathogens.