

CHAPTER I

INTRODUCTION

1.1 Background

Within the human body, there are multiple microbes involved in the gut or intestines, with one such microbe in the genus *Bifidobacterium* which is one of the first few microorganisms to colonize the human gastrointestinal tract (Jungersen, *et al.*, 2014). The genus *Bifidobacterium* is a Gram-positive, non-sporing, non-motile, anaerobic bacteria that is capable of producing lactic acid (O'Callaghan, and van Sinderen, 2016). *Bifidobacterium* is known to exhibit various beneficial effects such as being able to prevent or treat various ailments such as enteritis which is the inflammation of the small intestine (Chen, *et al.*, 2021).

The growth of *Bifidobacterium* within lab environments can be rather tricky due to several factors. This includes temperature due to heat and cold shock, oxidative stress, osmotic stress, and acidification. (Ruiz, *et al.*, 2013). Other studies such as Tkachenko *et al.* (2018), have also shown that other *Bifidobacterium* species that co-cultivated together showed increased the overall survivability of *Bifidobacterium* within their fermented milk product. *Bifidobacterium* species is one of the beneficial microorganisms present in our gut which is challenging to grow and utilize in fermented products due to it being unable to tolerate oxygen, thus requiring anaerobic conditions. However, within co-cultures, *Bifidobacterium* was seen to be more tolerant of oxygen which allows it to survive better. The benefits of co-cultures can be seen in the research of Tkachenko *et al.* (2018), where *Bifidobacterium* was co-cultivated with a specific strain of *Lactococcus lactis* ssp.

lactis where the genes for protecting against active oxygen species (specifically alkyl hydroperoxide reductase and Fe²⁺ transport system) were expressed during refrigerated storage which consequently improved the survival rate of *Bifidobacterium*. In this research the *Bifidobacterium animalis* strain was obtained from the Biology department in Universitas Pelita Harapan, with this strain being more aerotolerant compared to most of the other *Bifidobacterium* species being strict anaerobes. This strain was co-cultured with *L. acidophilus* and *L. fermentum* obtained from the Food Microbiology Laboratory in Universitas Pelita Harapan. *L. acidophilus* and *L. fermentum* are microbes found commonly within the lactic acid bacteria (LAB) group, which are often dominant in traditional fermented products such as sauerkraut, sourdough, kimchi, and many others (Icer *et al.*, 2023). As the LAB microbes do grow faster than *B. animalis*, hopefully the LAB species will be able to provide and support for the growth of *B. animalis*. There is potential in the uses of *B. animalis* along with *L. fermentum* and *L. acidophilus* within the food industry, most notably within yogurt making. These microbes are also well-known probiotics that can also benefit the overall health of the consumer by simply consuming them. The growth rate of the bacteria and co-culture are observed of each microbe to determine the ideal ratio of probiotics to form a co-culture.

1.2 Research Problem

There is some research currently that studies the effect of *Bifidobacterium* survivability within co-cultures but is usually limited to only one species of other bacteria and not more. The co-existence of several cultures may either have a positive influence or have a negative effect on the growth of the desired bacterium. Positive effects can include supporting growth of other microbes by helping remove

a substance like oxygen or emits a byproduct in which another species of microbes can use such as acids, the co-culture can thrive much better with the support of each species (Kim *et al.*, 2022). While there is minimal negative effects utilizing co-cultures, not all microbes can thrive together as some can outcompete other species either through resources or by making antibiotic substances (Baishya *et al.*, 2021).

The *Lactobacillus* strain also showed a very similar characteristic to *Bifidobacterium* thus making the selection of the inoculum within the co-culture mixture pose a challenge. In this research several selective media was also tested to selectively grow *Bifidobacteria* to find a method to isolate the microbe to calculate for the total *Bifidobacterium* count.

1.3 Objectives

1.3.1 General Objectives

The general objective of this research is to determine the optimal ratio of the prebiotic mixture and *Bifidobacterium* to increase the survivability rate.

1.3.2 Specific Objectives

The specific objectives of this research are:

1. To determine the growth rate of *Bifidobacterium animalis* subsp *lactis* BR2-5, *Lactobacillus acidophilus*, and *Lactobacillus fermentum*, and to identify the characteristics of the microbes through Gram staining.
2. To determine the best ratio of *Bifidobacterium animalis* subsp *lactis* BR2-5, *Lactobacillus acidophilus*, and *Lactobacillus fermentum* towards increasing the survival rate of *B. animalis*.