CHAPTER 1

1.1 Introduction

Bacterial cellulose (BC) is a three-dimensional structure made up of nanofibrils produced by aerobic bacteria such as *Acetobacter xylinum* (Silvia et al., 2017), *Gluconacetobacter xylinus* (Silvia et al., 2017), and *Komagataeibacter intermedius* (Lin et al., 2016). *K.intermedius* is first isolated from commercial vinegar. *K.intermedius* has a higher yield of BC when compared to *Komagataeibacter xylinus;* formerly known as *Gluconacetobacter xylinus,* which is another common strain that is used to produce BC (Fernandez et al., 2019). Fernandez *et al*.'s study proves that using *K.intermedius* to produce BC has the potential to be used industrially due to its high yield.

BC has been widely studied and used in the pharmaceutical, food and biomedical industries. BC is well known for its high-water retention, high crystallinity as well as great mechanical strength (Manoukian et al., 2018). It also has qualities such as being non-toxic, pure, permeable to liquid, biodegradable and biocompatible which is appealing to the biomedical industries in application for wound healing and local drug delivery. With those characteristics BC is able to act as a scaffold that is able to protect the wound from the outside environment and secondary infections as well as help the wound healing process by inserting compounds such as drugs, antimicrobial agents and even cells into its matrix (Portela et al., 2019).

Antibiotics are usually added into wound dressings to kill pathogenic bacteria at the site of wound (Negut et al., 2018). Repeated usage of products containing antibiotics could develop antibiotic resistance in the long run. Antibiotic resistance could complicate the treatments to otherwise simple bacterial infections, in this case the physician would need to look for another antibiotic that could work to cure the bacterial infection. This would take more time and is risky for the patient as the pathogenic bacteria will continue to proliferate inside the body. According to the World Health Organization antibiotic resistance is one of the biggest health threats globally. Per year there are at

least 700,000 deaths that are caused by antibiotic resistance (Willyard, 2017). To avoid antibiotic resistance the incorporation of probiotics has been suggested instead. There is evidence that probiotics are able to reduce the adverse effects of using antibiotics such as destroying the normal microbiota (Reid, 2006). The most well-known probiotics are strains from *Lactobacillus*, *Bifidobacterium,* some lactic acid bacterias and non-lactic acid bacterias such as *Saccharomyces cerevisiae* (Kechagia et al.,2013). *Saccharomyces cerevisiae,* otherwise known as yeast, has potential of usage in the medical field as a probiotic, even though it is well known for its use in the food industry. Yeast strains have also been extensively studied on their effect on gut health (Chzeruka et al., 2007). There are no studies done yet on whole viable yeast's probiotic effect on cutaneous wound healing.

Non-encapsulated probiotics show a decrease in viability and health benefiting effects when it is released to the environment of a wounded tissue (Saarela et al., 2000). Encapsulation is able to protect the probiotic from external factors such as acidity, oxygen as well as cell injury. By protecting it from external factors it is able to enhance its viability. Other than viability, encapsulation is also able to increase the probiotic's stability due to better binding with the fibrils inside the BC matrix (Huq et al.,2013).

The probiotic of interest *Saccharomyces cerevisiae* is a well-known probiotic but its knowledge on wound healing as well as encapsulation are still limited, which shows that this study is novel and is able to contribute to the field of study. *K.intermedius* adds to the contribution as well as novelty as most BC in the field of study are produced by *K.xylinus*. Using *Saccharomyces cerevisiae* as the probiotic and *K.intermedius* as the BC producing bacteria makes this topic novel and of significance in the field of research.

1.2 Research Question

The research question to be addressed in this study is as follows:

- Which encapsulation method is able to give the highest amount of *S.cerevisiae* loaded inside the BC?
- Would encapsulated *S.cerevisiae* exhibit antimicrobial activity ?

1.3 Research Objectives

The objective of this research is as follows:

- To determine the best way to encapsulate *S.cerevisiae* in bacterial cellulose.
- To measure encapsulated *S.cerevisiae*'s antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

1.4 Hypothesis

Based on the information gathered through literature review, the hypothesis is as follows:

- Injection-incubation method is the most effective method to encapsulate *Saccharomyces cerevisiae* and is able to exhibit an antimicrobial activity.
- Encapsulated *Saccharomyces cerevisiae* is able to exhibit an antimicrobial activity.

1.5 Scope of Research

The scope of research for this study is divided into several parts. The first part focuses on the optimization of adsorption-incubation (A-I) and injection-incubation (I-I) methods through modifications of the initial cell loading as well as the method of adsorption and injection. The optimized method with the best encapsulation results will be compared with the co-culture method (C-C) which is not able to be optimized. The best encapsulation method obtained from this round of comparison will proceed to be used to create probiotic BC which will be tested for its antimicrobial activity. The overall flow chart of this study will be shown in figure 1.1.

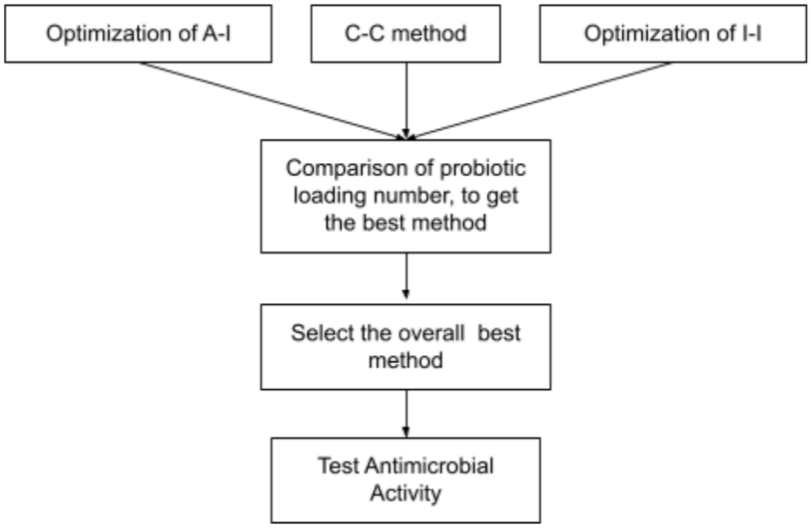

Figure 1.1 Flowchart of the overall study