I. INTRODUCTION

1.1 Background

Back-slopping is considered as the improvement method of the spontaneous fermentation process in fermented foods production (Smid *et al*., 2014). It involves inoculation of a small amount of previous successful fermentation product to the raw material (de Melo *et al*., 2022; Von Gastrow *et al*., 2020). This technique offers a more reproducible method compared with the natural fermentation as it shapes the microorganism diversity, thus decreasing the fermentation time which leads to cost efficiency. It has been widely used since ancient times for the production of traditional fermented products, including *dadih*, one of the oldest traditional fermented foods from Indonesia (Wirawati *et al*., 2019; Venema & Surono, 2019; Wirawati *et al*., 2021; Ramaiyulis *et all*., 2021). It is made by fermenting buffalo milk and is made in an artisanal manner. However, a drawback of the back-slopping method is that it may also support the growth of pathogenic bacteria as it is inoculated along with the useful microbes**.** The continuous back-slopping amplifies the accumulation of pathogens into the hazardous level, thus losing the fermenting culture activity and becoming harmful (Bintsis & Papademas, 2022; de Melo *et al*., 2022; Venema & Surono, 2019). Unfortunately, there is still a lack of studies assessing the safety of back-slopping methods despite its advantages for the artisanal product in the small-scale industry.

Staphylococcus aureus is one of the most important and prevalent food pathogens that is prone to contaminate *dadih* as it is the natural inhabitant in humans. As a traditional product made in an artisanal manner, the majority of the contamination source comes from the food handlers, although infrequently from the contaminated raw ingredients (Fetsch &

Johler, 2018). Moreover, as milk contains balanced and rich nutrients, it becomes a susceptible environment for the growth as well as transmission vehicle of *Staphylococcus aureus* (Campos *et al*., 2022). It has been explored that the use of natural antimicrobials that are already present in the food system can be utilized as a strategy to phase out typical preservation technologies (Rendueles *et al*., 2022). This field of knowledge is referred to as biopreservation.

Biopreservatives have gained a great attention in the scientific community and industry due to its potential as a promising preservation system (Silva *et al*., 2018). It utilizes microorganisms referred to as protective culture, that promotes a protective action from pathogenic or toxigenic microorganisms, thus increasing the safety and extending the shelf-life of the product. Moreover, protective culture refers to bacteria strains that are generally recognized as safe (GRAS). The protective action is the result of a wide range of bioactive compounds, such as bacteriocin, a ribosomally synthesized protein, antimicrobial peptide and short peptides that generate bactericidal or bacteriostatic activity (Wang *et al*., 2014). The main bacteriocin producing bacteria that act as protective culture are Gram-positive bacteria including lactic acid bacteria (LAB) and Bacillus. In the food industry, the most widely used and accepted bacteriocin as food additives by the World Health Organization and United States are natamycin and nisin, which is produced by LAB (To *et al*., 2022). However, the antimicrobial activity of LAB bacteriocin is affected by pH, temperature, composition, and natural microbiota composition in the food environment. Therefore, the utilization of Bacillus as the protective culture and its bacteriocin have harbored more interest as they exhibit broader antimicrobial activity compared to LAB, thus generating a

higher application in food, agricultural and pharmaceutical industry (Basi-Chipalu *et al*., 2022).

Bacillus strains are considered as the second most producer of bacteriocin after LAB (Sharma *et al*., 2021). Bacillus has been granted as GRAS by the Food and Drug Administration (FDA) of the USA, with several strains considered as industrially crucial and have a big potential to be utilized in the food industry. One of the strains being *Bacillus subtilis*. The bacteriocins produced by *Bacillus subtilis* are subtilin and subtilosin. It has been proved to inhibits the growth of a wide variety of Gram-negative bacterias such as *Escherichia coli* and *Pseudomonas aeruginosa*, as well as Gram-positive bacterias such as *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*.

Furthermore in this study, the protective culture used was made into the dry concentrated protective culture (DCPC) to ease the utilization and preservation. This was done as the protective culture was used for a period of time. Moreover, optimum activity of the microbes was expected to be higher as during the DCPC production, the growth and condition of the bacteria was strictly controlled

Thus, this study aimed to fill the gap by virtue of investigating the safety of back-slopping by assessing pathogen survival and further evaluating the protective action of *Bacillus subtilis* P5-6 to increase the control and/or eliminate the growth of the food pathogens in the fermented milk food system. Furthermore, conducting the production of dry concentrated protective culture of the *Bacillus subtilis*, ultimately escalates its potential uses as an effective biopreservative in the food industry.

1.2 Objective

The objective of this research are:

1. To identified the inhibitory activity of *Bacillus subtilis* P5-6 against *Staphylococcus aureus*
2. To produce a dry concentrated starter culture from *Bacillus subtilis* P5-6 cells
3. To investigate the safety of back-slopping methods by assessing the survival of food pathogen, conjointly the protective action of *Bacillus subtilis* P5-6 to increase the safety of traditional fermented milk products.

1.3 Research Scope

The scope of work of this research are:

1. Analyzed the inhibitory activity of *Bacillus subtilis* P5-6 against LAB and *Staphylococcus aureus*
2. Prepared concentrated protective culture in the form of concentrated powder and analyzed the minimum concentration needed to inhibit the growth of pathogenic bacteria.
3. Sample preparation, include raw material and consumable collection such as buffalo milk, bamboo tube and banana leaves. Followed by *dadih* fermentation to obtain the product.

4. Prepared the control and treatment condition regarding the aims of the project. Followed by performing back-slopping from control and variable batch.

5. Analyzed the viability of microbes, pH and titratable acidity in each fermentation batch.

    a. Enumerated the sample on MSA to detect the presence of *Bacillus subtilis* P5-6, MRS for LAB and BPA for *Staphylococcus aureus*.

1.4 Hypothesis

Pathogenic bacteria can survive in the back-slopping methods, but Bacillus as protective culture could exhibit antimicrobial activity towards the pathogen, thus increasing the safety of the back-slopped fermented milk.

1.5 Importance of Study

1. Contribute to the body of knowledge on back-slopping methods to increase the safety of traditional fermented milk, thus developing the industries of artisanal product

2. Contribute to the development of potential bacteria as a novel biopreservative in the form of protective culture for its usage in the food industry