CHAPTER 1

INTRODUCTION

1.1 Research Background

Soy sauce is a popular soybean-based fermented condiment in Asian countries. In the soy sauce industry, different types of soy sauce are available due to the variety in basic ingredients (soybean, wheat, salt, and water) ratio, as well as the duration of the fermentation process. According to Röling, Apriyantono & Van Verseveld (1996), soy sauce properties are greatly affected by the microbial diversity during the fermentation process. Despite having a lot of varieties, different types of soy sauce share the same two fermentation stages, which are *koji* and *moromi* fermentation. *Koji* is a solid-state fermentation of soybeans and wheat using mold spores (Devanthi & Gkatzionis, 2019). On the other hand, *moromi* is a fermentation step where the *koji* from the previous step get immersed in a brine solution for months (Devanthi & Gkatzionis, 2019). *Moromi* fermentation allows halophilic microbes to grow and produce the essential metabolites contributing to the aromatic and flavor profile of the end product. *Moromi* fermentation revolves around the indigenous lactic acid bacteria (LAB) and yeasts. The factors that affect microbial diversity of soy sauce are raw materials and fermentation conditions applied in the production (Devanthi & Gkatzionis, 2019). Traditional soy sauce production is conducted in a non-sterile environment, which results in spontaneous fermentation by indigenous microorganisms. Traditional soy sauce fermentation relies on the enzymes secreted by the starter cultures to break down proteins, lipids, and starches into peptides, free amino acids, volatiles, and saccharides (Diez-Simon et al., 2020). Thus, making the microbial community to hold an important role in constructing the end product's distinctive profile. Reportedly, there are two predominant halophilic microbes used in the soy sauce industry, *Tetragenococcus halophilus* (lactic acid bacteria) and *Zygosaccharomyces rouxii* (yeast), that were responsible for the product's distinctive properties. The lactic acid bacteria (LAB) grows at

the beginning of *moromi* fermentation, producing organic acids which cause the environment to be acidic (pH 4.0-5.0). Despite knowing the two predominant microbes' role in soy sauce production, the effects caused by other microorganisms in the microbial community cannot be neglected and the roles of the majority of them during the fermentation process remain unclear.

One of the commonly found bacteria in soy-based fermented foods are *Bacillus* spp. (Liu et al., 2015). In general, *Bacillus* is known to produce a variety of antimicrobial peptides which results in antimicrobial activity against a wide range of pathogenic microorganisms (Caulier et al., 2019). One of the commonly found bacteria in FSF is *Bacillus subtilis*, a gram-positive soil bacterium which has been widely used due to its protein secretion ability, fast growth, and its ability to produce endospores (Choi et al., 2020; Su et al., 2020). There are many enzymes that *B. subtilis* are able to produce in industries, which include amylases, xylanases, lichenase, cellulases, alkaline serine proteases, β-galactosidase, and a lot more (Su et al., 2020). These enzymes can be applied in many different industries including food industry due to the status given by The United States Food and Drug Administration (FDA), claiming that this species is generally recognized as safe (GRAS). Proteases produced by *B. subtilis* can be beneficial in preparation of soy protein hydrolysates (SPHs) and flavor profile of soy sauce (Su et al., 2020; Zhao et al., 2018). Protease enzyme in soy sauce production is responsible for its flavor profile as its role is to cleave the proteins into amino acids and peptides which lead to higher total nitrogen concentration and reduced bitterness (Kitano et al., 2002; Sandhya et al., 2005). There have been a few cases in which *B. subtilis* are found in soy sauce. In 2015, *Bacillus subtilis* EMD4 that exhibited strong antibacterial activity towards *B. cereus* ATCC14579 and *B. thuringiensis* ATCC33679 was isolated from Korean soy sauce (Liu et al., 2015). Not only naturally occurring in the soy sauce production process, some studies intentionally added *B. subtilis* to assist the fermentation process. In a study about utilization of engineered *B. subtilis* for soy sauce de-browning, it was found that *B. subtilis* is a part of the natural *moromi* microbial community as 18 out of 139 isolates are *B. subtilis* strains, which also indicated that these strains are capable of growing under soy sauce fermentation conditions (Det-udom et al., 2019).

Although there have been many cases about *B. subtilis* to be present during the soy sauce fermentation process, the studies do not talk in-depth about the role it has during the fermentation process, whether it causes significant changes in the fermentation process or not. As mentioned above, *B. subtilis* have many properties that can be beneficial for a wide range of industries. As of now, there has not been any studies that summarize and compile the role and advantages of *B. subtilis* in soy sauce production. Therefore, this study aims to address the role *B. subtilis* has and assess its safety aspect since several strains may cause detrimental health effects. This study will address the role and safety aspects of *B. subtilis* strains that have been previously reported whether it was naturally part of the microbial community or added during the *moromi* fermentation stage.

1.2 Objective

The objective of this research is:

To conduct a systematic literature review about *Bacillus subtilis* beneficial and harmful effects on soy sauce fermentation.

1.3 Scope of Work

The scope of the research of this thesis is:

1. Screening the published studies regarding *B. subtilis* and soy sauce production (purposely added to assist in production process or naturally occur during the fermentation).
2. Categorize and summarize the screened studies according to *B. subtilis* properties and its role in soy sauce production which includes potential harmful effects.
3. Evaluate and analyze both the beneficial and harmful effects of *B. subtilis* in soy sauce production for future references.