Chapter 1: Introduction

1.1 Background

In this past decade, the gut microbiome and beneficial bacteria were being studied due to the correlation to human and animal health and physiological function, such as nutrient absorption, synthesis of nutrient, immune modulation, defense against microorganism, and modify behavior (Trinh et al., 2018; Clarke et al., 2014; Honda and Littman, 2012). It has been known that proper sleep, reducing the used of steroid and non-steroid drug, and proper diet, especially eating numerous high fiber and prebiotic food such as grain, fruit, vegetable, seed, and beans able to rebalance the gut microbiome and bring homeostatic to the host (Smith et al., 2019; Ercolini and Fogliano, 2018; Rogers and Aronoff, 2016). Also, the consumption of beneficial microorganisms from prebiotic and probiotic able to rebalance the gut microbiome and improve human health (Azad et al., 2018; Suvorov, 2013).

Probiotics are microorganisms that come from various sources that able to adhere, survive in the host gut, and bring beneficial effects to the host (Markowiak and Śliżewska, 2017). Mainly, the word of probiotic comes from the Greek language has a meaning “for life”. The definition of probiotic was changed over and over until the Food and Agriculture Organization (FAO) of the United Nations World Health Organization (WHO) redefined the probiotic term to “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO working group, 2002; Hotel & Cordoba, 2001).

Numerous studies have shown that short term and long-term consumption of probiotic will increase beneficial microorganism in the gut, prevent dysbiosis (reduction of

pathogenic bacteria), increase oral cavity microbiome, and able to improve cardiovascular health (Gao et al., 2019; Khalesi et al., 2019; Dassi et al., 2018). Besides, the microorganism that is classified as probiotic able to digest nutrition, stimulating immune function, and inhibit pathogenic bacteria activities from causing harm to the host (Azad et al., 2018).

Recently, 3 types of microorganisms such as bacteria, mold, and yeast are commonly used for probiotic production. Those microbes come from 2 sources, a conventional source and an unconventional source (Sornplang and Piyadeatsoontorn, 2016). The conventional source refers to microorganisms that were isolated from a human source such as breast milk and fecal, and dairy product. Meanwhile, an unconventional source of probiotic comes from non-dairy fermented food products, non-human intestinal sources, and numerous parts of the digestive tract of animals (Rajoka et al., 2018; Pundir et al., 2013; Yanagida et al., 2006).

Unconventional source of probiotics were developed to provide probiotics for the lactose intolerant individual, therefore they can consume the probiotic without consuming the dairy product. Most bacteria being used in probiotic production come from the dairy that requires their prebiotic source, milk to be the reservoir for their living. Non-human intestinal and non-dairy fermented product has different in raw material and ingredients, this factor will lead to different microorganism species or strain that are available to be a candidate for probiotic (Sornplang and Piyadeatsoontorn, 2016; Schoster et al., 2014; Pundir et al., 2013; Ramirez-Chavarin et al., 2013; Siddiqee et al., 2012).

Isolating microorganisms from different animal gut such as chicken, dogs were shown to be a potential candidate for probiotic production for human (Rajoka et al.,2018; Beasley et al., 2006). However, there is little known of isolating microorganisms from insect gut to be a potential probiotic. Insects, especially bees, are one of the most important pollinators of

various plants that is important in preserving the wild habitat and world corps production (Baracchi, 2019). Most bee products such as honey, pollen, and wax are nutritional and numerous beneficial effect in medical use (Ajibola et al., 2012; Khan et al., 2007; Hargrove et al., 2004) Beside from bee product, study shown that giving probiotics isolated from bee gut able increase bee health thru enhancing their immune function and able reform the intestinal epithelium, especially when the bee was infected with a pathogen (Daisley et al., 2020; Killer et al., 2014; Pătruică et al., 2013; Audisio and Benítez-Ahrendts, 2011). Based on those studies, most of the bacteria were categorized as lactic acid bacteria (LAB) that are similar to current human probiotic (Olofsson et al., 2016; Asama et al., 2015; Aween et al., 2012; Kroyer and Hegedus, 2001). Thus, the bee microbiome can be a potential candidate to be used as a human probiotic.

The screening of probiotic should go several process, first it will go for in vitro study where microorganism was screen in the laboratory setting. Once in vitro study is conducted, then it goes to in vivo or animal model and once the animal study tells the probiotic or compound is beneficial and prophylaxis, it will go to the clinical trial. In all probiotic tests, 3 different criteria need to be passed, there are safety, functionality, and technology production (Markowiak and Śliżewska, 2017; Papadimitriou et al, 2015; Tsuda and Miyamoto, 2010, European Food Safety Authority (EFSA), 2005).

Currently, there are 2 research of probiotic potency that have been done in different bee gut species which taken from Indian honey bee (*Apis cerana indica*) and honeybee (*Apis mellifera*) (Kenfack et al., 2018; TR, 2018).

In this study, the author assesed the probiotic potency through in vitro study. 10 isolated bacteria consist of 5 *Lactobacillus kunkeei*, 1 *L. brevis*, 1 *L. casei*, 1 *Bacillus velezensis*,

1 *B. vallismortis*, 1. *B. pumilus*, and 1 *Bacillus sp.* that have been taken from 3 different bee gut (*Apis mellifera, Apis cerena,* and *Apis florea*) that life in Chiang Rai, North Thailand.

1.2 Objective

The main research in this study is to characterize the probiotic properties of 10 bacteria (5 *Lactobacillus kunkeei*, 1 *L. brevis*, 1 *Bacillus velezensis*, 1 *B. vallismortis*, 1 *B. pumilus*, and 1 *Bacillus sp.*) that have been isolated from different bee gut through several criteria :

1. Evaluate the safety of bacteria thru antibiotic resistance and hemolytic activity.
2. Elucidate the effect of human organ physiological stress such as lysozyme, acidic environment, pepsin, and pancreatin toward the viability of bacteria.
3. Evaluate the cell surface hydrophobicity, β – galactosidase activity, and autoaggregation of the bacteria.
4. Based on the overall test, bacteria that able to show the safest aspect, retain the viability towards different treatments, and shown the ability towards several testing will be pick as a potential candidate.