

## CHAPTER I

### INTRODUCTION

Foraging bees collect pollen from flowers in their hind legs in a distinctive scuttle then return to the hive and store it as bee bread to be consumed by adult bees and fed to the larvae. In the beginning, the bee bread consists of pollen, nectar and secretion from the bee's salivary gland. The pollens are accumulated into cells of brood comb which are finally seal with a drop of honey. Fourteen days later this bee pollen is chemically changed and, this change is considered to cause by the intervention of many microorganisms including lactic acid bacteria (LAB) and other microorganisms bacteria or yeasts (Vásquez, et al., 2009).

Bee pollen contain nutrients including proteins, sugars, lipids, fibre, mineral salts, amino acids, phenolic components and vitamins. It has been found that about eighteen amino acids are present in the pollen, among which proline, glutamic and aspartic acids, lysine and leucine, are prevalent that form about 55% of total amino acids. Bee pollen is loaded in polyphenol substances, mainly flavonoids that give it an importance to be used for human food (Krystyjan et al., 2015). The chemical content of bee pollen differs; it is affected by the environmental situations and the plant from which the pollen is collected. Render to its extended and multiple nutritional importance of bee bread, it is deemed as human diets in some countries and conserved by official quality standards. However, human beings cannot consumed pollen in a large amounts in their food, also is generally considered as an antiseptic additive. Bee pollen can be utilized as a pharmaceutical for handling different illnesses. Krystyjan et al., (2015) reported that the significant nutrients of bee bread could be used to supply energy for athletes. These researchers also suggested that pollen has a positive impact on chronic prostatitis.

Lactic acid bacteria (LAB) strains are considered as an unharmed nutriment class microorganisms in general and also used as probiotics to provide good health for human (Vásquez et al., 2012). The structure of bee bread vary chemically from that of bee pollen, bee bread has more acidity because of its content of lactic acid. The LAB and other metabolites present in bee pollen will maintain bee bread from spoilage for many

months (Vásquez et al, 2009). LABs are good source of antimicrobial substances like organic acids, hydrogen peroxide and antimicrobial peptides. There is an important difference in the production of antibacterial activities and many other useful features among the various species and genera within LAB (Forsgren et al., 2010). Additionally, LAB produced metabolites that have a wide inhibitory spectrum against pathogens (Olofsson et al., 2014).

Lactic acid bacteria (LAB) are present as flora inside humans, insects and animals. The strains within LAB have beneficial properties and they are usually present as both exogenous and endogenous bacteria in healthy humans (Vásquez, et al., 2012). Additionally, they play important roles in the industry of food and the fermentation of dairy, meat and vegetable products. Also, LAB genera are mostly distinguished as secure food bacteria and often used as probiotics that give good health to human. A probiotic is a culture of LAB that have useful health impacts for host when ingested in adequate amounts, but to serve as a probiotic the microorganisms is required to have the ability to tolerate the acidic condition (low pH) and resist bile acids (Erklölá et al., 2000).

Stingless bees (*Hymenoptera: Apidae: Meliponini*), eusocial insects which are vastly spread over the entire tropical and subtropical areas but some oceanic islands, are significant pollinators in Peninsular Malaysia. These kinds of bees exist in perennial settlements consisted of some hundred to many thousand workers and employ the resins from one hundred or more of the varied plant types for food, nest construction, and chemical defenses (Salim et al., 2012). Farming of stingless bee for their honey also known as “*kelulut*” is active in Malaysia. The honey is light in color and sourish in taste, which is different from other kinds of honey. In Malaysia, there are over 30 species of stingless bees (Ali et al., 2015). Studies on stingless bee honey locally known as “*madu kelulut*” were focused on the antimicrobial properties and isolation of LAB from the *madu kelulut* (Rasmussen et al., 2010). Vásquez et al. (2009 and 2012) confirmed the existence of LAB in bee bread. There is still a shortage of information relating to the types of LAB present in the bee bread of stingless bee farmed in Malaysia, their antimicrobial and probiotic properties. Bee bread is rich in nutrients and also LAB. It is expected that LAB with probiotic properties are present in bee bread, and it may be

possible to use the bee bread as a probiotic food supplement to promote human and animal health.

The purpose of the current study was to assess the probiotic properties of LAB isolated from bee bread produced by stingless bees in selected farms in Malaysia. Therefore, the objectives of this research were:

- i. To isolate LAB from bee bread of stingless bee *Thorasica* spp. from Sangai Merab/Kajang and Negeri Sembilan, *Itama* spp. from Sangai Merab, Kajang, Negeri Sembilan, Kedah and Terengganu and *Terminata* spp. from Negeri Sembilan.
- ii. To determine the probiotic properties (low pH and bile acid tolerance) and antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 13311 and *Escherichia coli* ATCC 25922.
- iii. To evaluate the antibiotic resistant patterns of the isolated LAB.