

## CHAPTER I

### INTRODUCTION

Honey is a thick, sweet liquid produced by bees from the nectar of flowers. (Al-Waili, 2004). The ancient Egyptians observed that honey not only a delicious food but also pointed out its use as a healing substance. Such concept is referred to in Surat El-Nahl, the Holy Quraan (Molan, 1992a & b). Honey has a unique nutritive composition including carbohydrates, water and more than 180 substances including amino acids, vitamins, minerals and enzymes (Ghoshdastidar & Chabrabarti, 1992; Weston & Brocklebank, 1999). Honey is a product with minimal types and level of microorganisms. However, it can carry spores of yeast, mold and bacteria, which may persist in it (Al Hindi, 2005). Honey can be used as an ingredient in food or as part of a nutraceutical, drug and cosmetic. Therefore, counts and types of microorganisms in honey need to be definitely recognized. The various bacterial and geographical origins play an important role on the characteristic of honey (El-Sherbiny & Rizk, 1979). Most microorganisms do not grow in honey because of its low water activity and high osmolarity. Therefore, it is difficult to isolate and grow microorganism from honey.

There are many studies on isolation of lactic acid bacteria from stomach of bee, flowers, and plants, but reports from honey are comparatively few. Akihito *et al.*, (2009) reported that they have isolated lactic acid bacteria from flowers and fruits, and identified them as *Lactobacillus kunkeei*, *Fructobacillus pseudoficulneus*, and *Fructobacillus fructosus*. Isolates of lactic acid bacteria, belonging to the *Lactobacillus*, *Lactococcus*, *Leuconostoc* genera were also isolated from flowers (Tavaria *et al.*, 2002), from plant surfaces and plant associated products. Some of these LAB were found to be antagonistic for strains phytopathogens against the bee larvae (Visser *et al.*, 1986). In addition, lactobacilli and *Bifidobacterium* were found in stomach of bee and shown to have antimicrobial properties against *Escherichia coli* and *Paenibacillus larvae*. These bacteria were reported to possess beneficial

properties for honey bee health specially American foulbrood (Eva *et al.*, 2009). LAB in the genera *Lactobacillus* and *Bifidobacterium* were also identified in bee pollen and bee bread (Eva *et al.*, 2009). In addition, *Lactobacillus* and *Bifidobacterium* strains have been isolated from fresh honey having water content about 18%. *Lactobacilli* and *Streptococcus* were found in honey (Hosny *et al.*, 2009; Ruiz & Rodriguez, 1975). *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Pediococcus* species were isolated from honey wine (Bekele *et al.*, 2006).

More than two thousand bacterial strains isolated from six US domestic honeys and two manuka honeys from New Zealand were screened for production of antimicrobial compounds (Lee *et al.*, 2008). They reported that a high incidence of antimicrobial inhibition determined by deferred inhibition assays was observed with the bacterial isolates from all eight honey samples. LAB also produce various antimicrobial compounds, which can be classified as low-molecular-mass (LMM) compounds such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), diacetyl (2,3-biutanedione), and high-molecular-mass (HMM) compounds like bacteriocins (Jay, 1982; Klaenhammer, 1988; Piard & Desmazeaud, 1991). There are many reports on the production of antimicrobial compounds by lactic acid bacteria (LAB) isolated from fruits and fermented food (Lindgren & Dobrogosz, 1990), but reports on LAB from honey are comparatively few. However, in medical practice nowadays antibiotics are most commonly used to treat infections. The extensive use of antibiotics has led to antibiotic resistant pathogenic bacteria becoming a big problem. People are, however, becoming aware about the side effects of these compounds. This fact has led to an interest in traditional medicine and drugs to find new treatment solutions and preventive treatments which are based on old wisdom.

So far in Malaysia some of the published papers related to honey: isolation and identification of phenolic acids in Malaysian honey with antibacterial properties (Aljadi & Yusoff, 2003), The effects of different types of honey on tensile strength evaluation of burn wound tissue healing (Rozaini *et al.*, 2004), antimicrobial activity of local Malaysian honey (Tumin *et al.*, 2005), the antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms (Tan *et al.*, 2009) and antibacterial properties of tualang honey and its effect in burn wound management:

a comparative study (Nasir *et al.*, 2010). No work has been done on the isolation and characterization of lactic acid bacteria from honey available in Malaysia.

Therefore, the objectives of this study were:

1. To isolate lactic acid bacteria from different types of honey commercial in Malaysia.
2. To screen lactic acid bacteria against multi antibiotic resistant strains of pathogenic bacteria using overlay method and micro titer plates.
3. To identify the LAB isolates that shows strong antibacterial activity by API 50 CH, 16S rDNA and RAPD-PCR analysis.

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