CHAPTER I

INTRODUCTION

Honey is a natural substance produced by honeybees from the nectar of flowers and from the excretion of living parts of plants, which the honeybees collect, transform, and combine with specific substances of their own, and then store and keep in the honey comb to ripen and mature (Olaitan et al., 2007; Omafuvbe & Akanbi, 2009; Kahraman et al., 2010; Estevinho et al., 2011). Islam recommends the use of honey as a spiritual food and medicine, and named an entire chapter in the Holy Qur'an called Surah al-Nahl (Molan, 1992b). Honey has been used as medicine in many cultures and regions for a long time (Quinn et al., 1994). The modern science has made it possible to specify their medical significance as bactericidal, bacteriostatic, antiviral, antifungal, antioxidant, anti-inflammatory, and antitumor (Molan 2001b; Lusby et al., 2005; Bardy et al., 2008; Estevinho et al., 2008). Honey possesses inherent antimicrobial properties, some of which are due to high osmotic pressure/low water activity, in which the low water activity of honey is considered as an inhibitory to the growth of the majority of bacteria and many yeasts and moulds. When the honey applied to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and could minimize bacterial growth (Molan, 1995b). In addition, honey has been employed to shorten the duration of diarrhea in patients with bactericidal gastro-enteritis due to bacterial infection (Haffejee & Moosa, 1985).

There are many studies on isolation of lactic acid bacteria (LAB) from stomach of bee, flowers, and plants. Endo et al. (2009) isolated lactic acid bacteria from flowers and fruits, and identified them as *Lactobacillus kunkeei*, *Fructobacillus pseudoficulneus*, and *F. fructosu*. *Lactobacillus kunkeei* is an inhabitant of fructose-rich niches and is a potential member of the fructophilic lactic acid bacteria, the genus

Fructobacillus comprised of four species namely, *F. fructosus*, *F. durionis, F. ficulneus* and *F. pseudoficulneus* (Endo & Okada 2008). These four species were isolated from a flower in South Africa (Endo et al., 2011). LAB in the genera *Lactobacillus* and *Bifidobacterium* were isolated from bee pollen and bee bread (Forsgren et al., 2010).

Recently, the use of medical implants such as catheters, pacemakers, joint replacement, prosthetic heart valve, silicone voice prostheses, end tracheal tubes and cerebrospinal fluid shunts has increased dramatically, these devices can become colonized by microorganisms which form biofilm and this biofilm leads to acute disseminated infection (Kojic & Darouiche, 2004). The majority of implant infections are commonly caused by the pathogenic *Candida* spp.

Biofilm is a principal form of microbial growth and is critical to development of clinical infection. Biofilm cells are more resistance to antifungal drug than planktonic cells (Beauvais & Muller, 2009). Biofilm formation is characterized by initial adherence of fungal cells to a substrate (either abiotic or mucosal surface) followed by germination, micro-colony formation, filamentation, monolayer development, proliferation and maturation (Finkel & Mitchell, 2011). Extra cellular matrix (ECM) is a defining characteristic of fungal biofilm, providing the cells protection from hostile factors such as host immunity and antifungal agents (Ramage et al., 2009). The majority of human mycoses are caused by opportunistic fungi (Boukraa et al., 2008). *Candida albicans* is a fungus which can grow on warm and moist surfaces and causes superficial diseases such as oral and vaginal thrush and chronic mucocutaneous candidiasis (Molero et al., 2010).

LAB are a group of beneficial microbes. They are one of non-pathogenic bacteria that play important role in our everyday life from fermentation, preservation and production of useful foods, and vitamins to prevention of specific diseases and cancer

due to their capability as an antimicrobial function (LeBlanc et al., 2011; Capozzi et al., 2012; Shah & Prajapati, 2014). LAB generate produced a variety of antimicrobial compounds and effective substances such as lactic, acetic, antibiotics, bacteriocins as well as hydrogen peroxide and carbon dioxide these compounds can inhibit the growth of *Candida* spp. (Ouwehand & Vesterlund, 2004; Schnürer & Magnusson, 2005; De Keersmaecker et al., 2006; Sathe et al., 2007; Hasslöf et al., 2010).

Rönnqvist et al. (2007) reported that a LAB isolate, *L. fermentum* Ess–1 exhibited activity against *C. glabrata* and *C. albicans*. Okkers et al. (1999) showed that *L. pentosus* produced a bacteriocin-like peptide, pentocin which had a fungistatic effect against *C. albicans*. Strus et al. (2005) reported that *L. debrueckii* showed strong inhibitory activity against *C. albicans.*

Candida spp. are recognized as one of the major causes of hospital-acquired infections. They can form biofilms on medical devices such as pacemakers, joint replacement, prosthetic heart valve, silicone voice prostheses and urinary or intravascular catheters which can cause device failure. The growing numbers of pathogenic fungi have become more resistance to known antifungal agents and to solve this problem, the purpose of this research was to isolate and identify lactic acid bacteria in honey, and evaluate their ability against five strains of *Candida* spp. namely, C. *albicans* ATCC1405, *C. glabrata* ATCC2001, *C. tropicalis* ATCC750, C. *parapsilosis* ATCC22019 and C. *krusei* ATCC6258. This study would use LAB as biological control instead of antifungal agents to reduce fungal diseases and inhibit pathogenic *Candida* species infections. Therefore, the objectives of this study were:

1) To isolate lactic acid bacteria from natural honey and identify them using API 50CHL assay and 16S rDNA.

2) To screen the LAB isolates for their antifungal properties against *Candida* species using overlay method and well method.

3) To determine the anti-adhesive activity of LAB against *Candida* spp. using the pre-coating and co-incubation experiments.